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INFLUENCE OF CERTAIN COLOR MUTATIONS ON BODY SIZE IN MICE, RATS, AND RABBITS

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INTRODUCTION

THIS paper is a further contribution to a series of studies on size inheritance in mice begun some years ago at the Bussey Institution of Harvard University by my colleagues and myself and designed to throw light on the question how particular mutant genes affect the growth of the body and so influence adult body size.

In previous studies it was found that the gene for brown pigmentation and the gene for dilute pigmentation accelerate growth and thus increase adult body size, as judged by criteria of body weight, body length, and tail length. By like criteria it has been found that the gene for short-ear and the gene for pink-eye decrease adult body size, but that the gene for agouti and the gene for complete albinism are neutral, neither increasing nor decreasing body size.

The present study is concerned with the influence on body size of three other mutant genes, leaden, pink-eye 2, and yellow. Leaden is phenotypically similar to blue dilution, and pink-eye 2 is phenotypically similar to ordinary pink-eye, but both are entirely distinct genetically from the better known mutants which they resemble. Leaden, unlike blue dilution, has a retarding influence on growth, and pink-eye 2 has a stronger retarding effect than ordinary pink-eye, as will presently appear. Yellow, as is well known, has a lethal effect when homozygous but increases body size when heterozygous.

This paper deals also with a further study of the influence of the brown mutation on body size, the investigation being extended from mice to rats and rabbits also.

THE INFLUENCE OF LEADEN ON BODY SIZE IN MICE

A stock of leaden mice (MURRAY 1932) was obtained from the ROSCOE B. JACKSON MEMORIAL LABORATORY, and leaden males were crossed with females of a black race (C 57 black) obtained from the same source. The leaden mice were homozygous for brown as well as leaden[2] (*bb ll*), the black

[1] Assistance in this investigation was rendered by the personnel of W.P.A. Project O.P. No. 465-03-3-630. Financial aid was given by a grant from the CARNEGIE INSTITUTION OF WASHINGTON, and laboratory facilities were provided by The Veterinary Science Laboratory of the UNIVERSITY OF CALIFORNIA.

[2] By a clerical error in the preparation of this paper the abbreviation *l* was used for leaden instead of the approved one, *ln*. Correction of the error in the proof would involve many changes

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race being homozygous for the corresponding dominant alleles (*B B L L*). Consequently the F_1 mice were black in color, like their mothers, but were double heterozygotes (*B b L l*). F_1 females were backcrossed to brown leaden males (*b b l l*), producing four genotypes of which the following numbers were reared. Black (*B b L l*), 210; brown (*b b L l*), 170; black leaden (*B b l l*), 114; brown leaden (*b b l l*), 111.

If *b* and *l* are not linked, it is to be expected that the four phenotypes will be numerically equal. But if they are linked, the crossover or recombination classes (brown and black leaden) should be smaller than the non-crossover classes (black and brown leaden). The difference observed between the two groups is not of statistical significance (less than three times the P.E.), and so we may conclude that linkage does not exist, which agrees with findings made at THE JACKSON MEMORIAL LABORATORY.

But the four phenotypes differ in survival value under the conditions of the experiment, which were as follows. Five or six F_1 females were caged with a single male, and no attempt was made to keep track of individual litters. Often there were litters of different ages growing up in the same cage. The cages were inspected at intervals of a week or ten days, and all young of weaning age were removed and recorded, the sexes being separated and reared apart to the age of six months, when they were weighed, chloroformed, and measured as to body length and tail length by Sumner's method. The relative survival values of the four classes are black 100, brown 80.1, black leaden 50.4, brown leaden 50.3. The relative survival value of non-leaden as compared with leaden is as 100 to 59.2. Evidently leaden individuals are under a handicap in competition with non-leaden litter mates. The observations made are not complete enough to show whether this handicap is effective before birth or only afterward. Even when leaden individuals survive, they attain a significantly smaller body size than their non-leaden litter mates, as the data summarized in table 1 show. Consequently we must conclude that this mutation like most others (but not all) is a disadvantageous one.

It will be observed in table 1 that brown individuals, even though they seem to have less survival value than blacks, nevertheless attain a slightly greater body size when they are heterozygous for leaden. This is true for both sexes and by all three criteria of body size—namely, weight, body length, and tail length. The differences in average size (next to the last column of table 1), it is true, are too small to have statistical significance in the number of observations made, except as regards the body length of females. But the regularity with which the differences occur indicates a

both in the text and in the tables, and it was decided to let the error stand, hoping it would not result in permanent derangement of terminology.

TABLE 1

Average body size in mice of four different genotypes resulting from a cross involving leaden. Weights are in grams, body lengths and tail lengths in mm.

	BLACK		BROWN		BLACK LEADEN		BROWN LEADEN		ALL NON-LEADEN		ALL LEADEN		BROWN > BLACK	LEADEN < NON-LEAD.
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	DIF. MEANS	DIF. MEANS
Weight, males	84	32.66±.25	83	32.84±.28	64	31.17±.22	57	31.06±.21	167	32.74±.19	121	31.12±.15	.18±.37	1.62±.24
Weight, females	116	25.07±.19	84	25.71±.22	45	24.14±.27	62	23.56±.25	200	25.45±.16	107	23.73±.16	.64±.29	1.72±.35
Body, males	94	100.08±.18	84	100.41±.18	64	99.58±.19	57	98.91±.22	178	100.22±.13	121	99.26±.14	.33±.25	.96±.19
Body, females	116	95.88±.21	86	96.98±.26	50	95.62±.35	54	95.09±.30	202	96.35±.17	104	95.40±.22	1.10±.33	.95±.27
Tail, males	89	82.76±.23	75	83.14±.22	54	80.76±.26	51	80.39±.25	164	82.91±.16	105	80.58±.18	.38±.31	2.33±.24
Tail, females	110	81.76±.20	83	82.29±.27	48	78.94±.32	48	78.52±.30	193	81.99±.16	96	78.81±.22	.53±.33	3.18±.27

TABLE 2

Average body size in mice of four different genotypes resulting from a cross involving pink-eye 2.

	BLACK		BROWN		p_2 BLACK		p_2 BROWN		ALL DARK EYED		ALL PINK EYED		BROWN < BLACK	p_2 < NON-p_2
	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	NO.	MEAN	DIF. MEANS	DIF. MEANS
Weight, males	318	33.81±.14	306	33.44±.12	199	32.09±.15	209	31.84±.14	624	33.63±.09	408	31.96±.10	.37±.18	1.67±.13
Weight, females	291	25.12±.12	275	25.08±.13	196	23.62±.13	214	23.03±.13	566	25.08±.09	410	23.32±.09	.04±.17	1.76±.13
Body, males	320	96.66±.11	308	96.40±.09	200	95.16±.14	208	94.52±.14	628	96.57±.08	408	94.83±.10	.26±.14	1.74±.13
Body, females	292	89.88±.11	282	89.48±.14	200	87.93±.16	220	87.42±.16	574	89.69±.09	420	87.66±.11	.40±.18	2.03±.14
Tail, males	306	88.35±.15	296	88.39±.14	183	85.64±.17	197	86.10±.17	602	88.36±.10	380	85.89±.12	−.04±.20	2.47±.15
Tail, females	280	84.84±.14	272	84.68±.13	192	82.29±.19	215	81.42±.17	552	84.76±.10	407	81.83±.12	.16±.19	2.93±.15

consistent influence of the brown mutation in increasing body size in this as in other mouse crosses.

Nevertheless when blacks and browns are homozygous for leaden, the blacks are as consistently larger than browns as they are smaller when only heterozygous for leaden. Again it must be noted that the differences are not statistically significant, yet the consistency with which they occur in both sexes, and by all three criteria, indicates a trend to larger size in blacks than in browns when both are homozygous for leaden.

Considering simultaneously the survival value of the several genotypes produced in this experiment and their respective body sizes, it would seem that brown, in this cross as in those previously studied, has a tendency to increase body size when only a single leaden gene is present, though at the same time the survival value of the brown heterozygotes is less than that of the blacks. But when the leaden gene is twice represented in the genotype, browns not only have less survival value than blacks but they are actually smaller in body size than blacks. In other words, the leaden gene is disadvantageous in combination with the brown gene even in heterozygotes, as indicated by diminished survival value, but in homozygotes there is added to this a diminution of body size. Consequently it seems clear that the brown mutation, which by itself has no harmful effects in cage bred mice and actually increases body size, nevertheless, when associated with the leaden gene, decreases survival value in heterozygous leaden individuals and also decreases body size in homozygous leaden individuals.

If we compare the body size of all non-leaden with that of all leaden individuals of the same sex (table 1, last column), we observe in every case differences of statistical significance, ranging from four to twelve times the P.E. This shows conclusively that the leaden mutation has a distinctly retarding influence on growth of the body.

THE INFLUENCE OF PINK-EYE 2 ON BODY SIZE IN MICE

A stock of mice homozygous for pink-eye 2 was kindly supplied by Dr. Elmer Roberts who discovered this mutation. This mutation was discovered in a wild caught individual, and Roberts informs me that the stock which I received had been derived from a subsequent outcross to wild stock made to remove impaired fertility in his stock of the *p*2 race. Strong evidence of their wild origin is obvious in the behavior of the descendants of the *p*2 mice which I received, but it is more conspicuous in the pink-eyed than in the dark-eyed descendants, which suggests that the *p*2 gene has a direct physiological action affecting behavior. Certain pink-eyed individuals have been noted in my records as "jumpers," because they refused to remain quiet on the weighing scales long enough for one to ob-

serve their weight. Frequently they would turn back-somersaults and then scurry away to a corner of the room. I have never observed comparable behavior among the dark-eyed litter mates of such pink-eyed jumpers. One would expect to find such individuals as a consequence of a cross-over, if a gene linked with pink-eye were responsible for the behavior, but no such animals are expected if the pink-eye gene is responsible for the jumping behavior.

In his paper describing the *p2* mutation, ROBERTS (1932) says "so far a non-agouti in the new mutation has not been found." This indicated the location of the new gene to be in the same chromosome as agouti. In a subsequent publication ROBERTS and QUISENBERRY (1935) reported obtaining non-agouti individuals and estimated the crossover percentage between *p2* and *A* at 21.2 percent in females and 19.6 percent in males.

Most of the animals which I received from ROBERTS were non-agouti browns (*a a bb*), but one was *A a bb* which in crosses with blacks produced gray as well as black F_1 offspring. All other *p2* males crossed with females of the C 57 black race produced only black F_1 offspring, which were vigorous, long-lived, and highly fertile. F_1 black females were now back crossed with *p2* males, about six females being kept in a cage with a single male. As in the experiments with leaden, no attempt was made to keep track of individual litters.

From the start it was observed that at weaning time dark-eyed individuals were more numerous than pink-eyed, which would seem to be due to their greater vitality, since theoretically the numbers of dark-eyed and pink-eyed individuals should be equal, the *p2* mutation, in my experience as in that of ROBERTS, being a simple recessive. Four phenotypes result from the back-cross—namely, (1) black, (2) brown, (3) *p2* black, and (4) *p2* brown—which are expected to be numerically equal. The respective numbers recorded at weaning time were black 415, brown 399, *p2* black 254, *p2* brown 282. Combined, the totals are 814 dark-eyed to 536 pink-eyed, a ratio of 100:65. The corresponding numbers of dark-eyed and pink-eyed individuals reared to maturity were 1202 and 828, a ratio of 100:68, which indicates survival value subsequent to weaning approximately equally great among pink-eyed and dark-eyed classes. This population included not only the survivors of the animals recorded at weaning time but also a considerable number of young not classified at weaning. Accordingly the exact survival rate subsequent to weaning cannot be precisely stated, but it was obviously no smaller among pink-eyed than among dark-eyed. Whatever handicap exists against zygotes homozygous for the *p2* gene therefore operates during either gestation or lactation, not subsequently.

The question whether the *p2* gene influences body size when in a hetero-

zygous condition, as in the F_1 parents and in their dark-eyed offspring, has not been directly investigated. The data obtained show only the relative body size of animals homozygous and heterozygous, respectively, for the *p2* gene (and genes possibly associated with it in the same chromosome), such animals being in other respects of identical genetic constitution.

The data are summarized in table 2. They show that pink-eyed individuals are in both sexes significantly smaller bodied than their dark-eyed sibs. The difference is in the case of males 11 times the probable error in weight, 13 times the probable error in body length, and 15 times the probable error in tail length. Among females the difference in weight is 13 times, in body length 14 times, and in tail length 18 times the probable error. There can accordingly be no doubt that the *p2* gene when homozygous acts as a depressant on growth, reducing body size.

One surprising result emerges from this study. The brown gene in all our previous studies except that with leaden had been found invariably to increase body size when homozygous. In the case of leaden, homozygous browns were larger bodied than blacks when leaden was present in single dose, but when leaden was present in double dose (homozygous), browns were of smaller size than their black sibs. In this cross browns are inferior to blacks not only in survival value but also in body size in the presence of either a single or a double dose of *p2*.

The two mutant genes, *l* and *p2*, are alike in having a retarding influence on growth and adult body size. This influence is manifested first in a reduced survival rate under competition with other genotypes and secondly in reduced body size of surviving individuals. The mutant gene brown, which has been found to increase body size in other genetic combinations, has in combination with the genes *l* and *p2* an interaction unfavorable to growth and survival, and the average body size of adults is reduced.

In an earlier paper, CASTLE et al. (1936) the interaction between brown and dilution was shown to be favorable, genotypes homozygous for both mutant genes being of larger body size than those which were homozygous for either one of them separately. The present experiment shows a different kind of interaction resulting in a genotype inferior to either one acting alone. Brown, which is ordinarily favorable (accelerating growth), in association with homozygous leaden (or homozygous pink-eye 2) becomes unfavorable, producing a genotype inferior to the action of either constituent gene acting by itself.

INFLUENCE ON BODY SIZE OF THE DOMINANT GENE FOR YELLOW COAT (A^y)

Through the kindness of DR. L. C. DUNN I received in 1938 a stock of inbred yellow mice from which I selected a male of the constitution

A^{y}a BB to cross with females of LITTLE's well-known *d br* race, which is *aa bb* in constitution. The F_1 progeny were of two genotypes, (1) *A^{y}a Bb* (yellow) and (2) *aa Bb* (black). These constitute what I shall call population 1, table 3. Males and females were kept in separate cages until they were six months old, when they were weighed, chloroformed, and measured as to body length and tail length. Two successive lots of young were thus reared, and their measurements are recorded separately under (a) and (b) and also combined in a total.

Certain of the F_1 yellow females were reserved for further breeding and were mated to males of the inbred strain C57 black obtained from THE ROSCOE B. JACKSON MEMORIAL LABORATORY. This cross also produced young of the two phenotypes yellow and black, which constitute population (2) of table 3, where the means of their weights, body lengths, and tail lengths are recorded.

Again certain of the F_1 yellow females were reserved for further breeding, some of them being homozygous for black (*A^{y}a BB*), others heterozygous for black (*A^{y}a Bb*), the two classes being scarcely separable except by breeding tests. These yellow females were mated to males of an inbred strain of ROBERTS' pink-eyed race of the constitution *a a b b p2 p2*. The progeny form population (3) of table 3. They were of two phenotypes, yellow and black, when the F_1 mothers were homozygous for black as in the lot 3a of table 1, but of three easily recognized phenotypes, yellow, black, and brown in the ratio 2:1:1, when the mothers were heterozygous for black as in lot 3b. These last animals were chloroformed and measured when a month or two younger than lot 3a, so the data for the two cannot properly be combined.

Also a few black females of population (2) were mated with *p2* males, producing two phenotypes, black and brown, which together constitute population (4), table 3.

From a study of these populations and a comparison of their constituents, we should be able to learn what effect, if any, the *A^{y}* gene has on body size, whether or not homozygous browns differ in body size from heterozygotes with black, and finally what effect *p2* has on body size in combination with black or with brown.

In populations (1), (2), and (3) we may compare the body size of yellow individuals with blacks of like parentage reared with them in the same cages, the two types differing only as regards a single gene, yellows being *A^{y}a* and blacks *aa* in constitution. In weight yellows are invariably much heavier than their black sibs of the same sex. Comparative average weights for male yellows and blacks, sibs reared together under identical conditions, are 48.1:36.8; 49.4:37.2; 48.9:37.1; 53.1:38.6; 50.2:40.3; 46.1:34.0. For females, comparative average weights are 49.6:29.2; 46.0:27.5; 46.9:

28.2; and 41.5:27.7. Yellow males are one-third (33 percent) heavier than their black brothers. The difference is even greater in the case of females, yellow females being 62 percent heavier than their black sisters. That the yellow gene has a greater effect in females than in males in producing adiposity was observed by DANFORTH (1927).

The greater weight of yellow individuals is due chiefly to their accumulation of fat, as was also shown by DANFORTH. But fatness is not the only difference. The data of table 3 show that the body length and tail length of yellows are also greater than the corresponding measurements of their black sibs. In every instance yellows are longer bodied than blacks where

TABLE 3

Comparative body size of yellow, black, and brown mice derived from crosses of yellow mice with three other color varieties.

MALES	YELLOW				BLACK				BROWN			
POPULATION	NO.	WEIGHT	BODY	TAIL	NO.	WEIGHT	BODY	TAIL	NO.	WEIGHT	BODY	TAIL
1a	15	48.1	104.4	89.0	18	36.8	101.2	87.4				
1b	19	49.4	103.2	87.3	30	37.2	101.1	86.6				
Total	34	48.9	103.8	88.3	48	37.1	101.1	87.0				
2	22	53.1	105.0	89.6	24	38.6	102.4	87.2				
3a (6 mos.)	21	50.2	106.1	91.5	24	40.3	103.2	89.3				
3b (4–6 mos.)	15	46.1	100.3	91.7	12	34.0	97.6	91.6	10	34.8	98.8	93.9
4 (4–6 mos.)					21	30.5	95.7	91.6	13	30.4	96.9	92.1
Females												
1a	8	49.6	100.9	81.1	10	29.2	95.7	81.8				
1b	23	46.0	101.1	87.1	14	27.5	95.3	85.0				
Total	31	46.9	101.1	85.3	24	28.2	95.5	83.6				
2	15	41.5	100.4	85.3	28	27.7	97.2	86.2				

significant numbers have been measured. Yellow males are on the average 2.6 percent longer bodied than their black brothers. Yellow females are 4.9 percent longer bodied than their black sisters. Tail-length is a less satisfactory measurement but shows yellow males 1.5 percent longer tailed than black males, while yellow females show only a fraction of a percent (0.2) longer tails than their black sisters. The consistency of the greater size of yellows is striking, though the differences are not very great.

It may be suggested that the greater body length of yellow mice is perhaps an indirect consequence of fat production, fat in the intervertebral disks forcing the vertebrae farther apart. Therefore it seemed desirable to measure individual long bones of the leg as well as total body length. For this purpose femur and tibia of the legs of 45 male yellow mice were cleaned, dried, and compared with the corresponding bones of 25 male black mice, brothers of the yellows reared in the same cages with them.

The respective average femur lengths are for yellows 15.075 ± .032 mm, for blacks 14.996 ± .053 mm. The difference is .079 ± .062 mm. For the tibia lengths of the same mice, the average for yellows is 18.252 ± .039 mm, and for blacks 18.072 ± .064. The difference in this case is .180 ± .082 mm. In neither case is the difference statistically significant, but it agrees with the observations on body length and tail length, indicating that the greater dimensions of yellows were not due to a padding of fat between vertebrae but to greater size of the individual bones.

The data on the influence of the brown gene, when homozygous, on body size in this cross are scanty, being recorded only for males in crosses

TABLE 4

Percentage of change in body size induced by certain gene combinations.

GENES	WEIGHT	BODY	TAIL
bb	+ 4.27	+1.51	+1.30
dd	+ 2.10	+0.90	+2.64
bbdd	+ 5.81	+2.70	+3.89
pp	− 1.01	−0.14	−0.72
bbLl	+ 1.07	+0.71	+0.55
Bbll	− 3.64	−0.61	−2.94
bbll	− 5.47	−1.00	−3.42
bbP2p2	− 0.70	−0.36	−0.07
Bbp2p2	− 5.53	−1.89	−4.04
bbp2p2	− 5.90	−2.46	−3.29
A^{v}a ♂♂	+33.00	+2.60	+1.50
A^{v}a ♀♀	+62.00	+4.90	+0.20
aa	0	0	0
cc	0	0	0

3b and 4, table 3. They indicate a probably slightly greater body size of homozygous brown males than of their heterozygous black sibs, as commonly observed in other mouse crosses.

SUMMARY ON THE EFFECTS OF MUTANT GENES ON BODY SIZE

From a study of data presented in this paper and from the data contained in previous papers table 4 was compiled to show the average change in body size induced by certain mutant genes singly or in combination. In obtaining these results, both sexes were weighted equally. It will be noted that the action of two genes, *b* (brown) and *d* (dilution), when homozygous and acting singly, is distinctly favorable to growth, but that three other genes, *p*, *l* and *p2*, diminish body size, while two genes, *a* and *c*, are without observable effect on body size. The favorable genes, *b* and *d*, in combination have a cumulative favorable action on body size greater than that of either by itself. The effect of gene *b* has also been studied when it is asso-

ciated with the unfavorable genes, *l* and *p2*. Surprisingly, it is found that the unfavorable action of *l* and *p2*, instead of being diminished by interaction with *b*, is increased by it. The gene *l* when heterozygous almost (but not quite) overcomes the favorable action of homozygous *b*, in the combination *bbLl*, the net effect of which is slightly plus. When gene *l* is homozygous and *b* heterozygous, in the combination *Bbll*, the net result is a strongly minus influence. When both genes are homozygous, in the combination *bbll*, the net result is still more strongly minus.

The interaction of gene *p2* with *b* is similar to that of *l* but more strongly unfavorable, since *p2* exerts a minus influence even when heterozygous in the combination *bbP2p2*, and the net result is more strongly minus in combinations in which *p2* is homozygous.

The mutant gene A^v, lethal when homozygous, increases body size when heterozygous, and the action is stronger in females than in males.

INFLUENCE OF THE BROWN AND THE BLUE MUTATIONS ON BODY SIZE IN RATS

Since it has been shown in earlier publications that in mice the color mutations brown and blue act as accelerators of growth and so result in increased body size, it will be of interest to inquire whether similar action is exercised by what are apparently homologous mutations in rats. A cinnamon mutation in rats has been described by DR. HELEN DEAN KING (1932), who observed its occurrence in a colony of wild caught gray rats reared in captivity at THE WISTAR INSTITUTE. Cinnamon, as in mice, is the agouti genotype, the non-agouti genotype (*aa bb*) being called chocolate or brown. As in mice, cinnamon is recessive to gray and chocolate recessive to black. DR. KING generously supplied a stock of cinnamon rats for these experiments, and I obtained from them the chocolate combination.

The blue mutation of rats was discovered by DR. ELMER ROBERTS. Descendants of stock distributed by him were generously supplied for this investigation by DR. P. W. GREGORY of the COLLEGE OF AGRICULTURE at Davis, California. The blue dilution of rats, like that of mice, is recessive to ordinary intense pigmentation.

It was my plan to test simultaneously the influence of the cinnamon mutation and the influence of the blue mutation in rats by crossing cinnamon with blue and observing the relative body size of the segregating color classes in later generations. The F_1 individuals produced in this way were gray in color like wild rats, the cinnamon and the blue mutations of the parents being alike recessive and complementary. An F_2 population was next produced consisting of 221 individuals of which 137 were gray, 35 cinnamon, and 48 blue. The expectation based on a 9:3:4 ratio would be

124:41:55, from which the observed numbers do not deviate significantly.

It will be noted that only three phenotypes are mentioned—namely, gray, cinnamon, and blue. Theoretically the blue class should include both blue-grays and blue-cinnamons in the ratio 3:1, and there is reason to think that it actually had this constitution. But the two sorts of blues are so similar in appearance that it is impossible in many cases to distinguish between them without breeding tests, which for the purposes of the experiment were considered unnecessary.

F_2 individuals taken at random from the blue class were now backcrossed reciprocally with F_1 grays which were double heterozygotes (*Bb Dd*). When the blue parent was a homozygous blue-gray (*BB dd*), only gray and blue offspring were to be expected and in equal numbers. But if the blue parent were either heterozygous or homozygous for brown (*Bb dd* or *bb dd*), then half the offspring would still be expected to be blue as in the previous case, but the non-blues would be expected to be divided between grays and cinnamons, either as 3:1 or as 1:1.

No attempt was made to classify on this basis the blue parents, since the prime object of the experiment was to ascertain the relative body size of blues as compared with non-blues, and of cinnamons as compared with grays, when these occurred as litter mates.

Accordingly the backcross litters at weaning time (age about one month) were separated as to sex but not as to color, about six or eight individuals being kept together in a cage and supplied with Purina dog-chow and water. At the age of four months the animals were weighed and destroyed. Measurements of body length and of tail length were not made as in the mouse experiments, partly because these seemed unnecessary in view of the unexpected result with the weights and partly for lack of time. Full adult weight had of course not been attained at the age of four months, but it was thought on the ground of preliminary observations that growth would at this age be sufficiently advanced so that relative final body size of the three phenotypes would be evident.

The backcross population reared to the age of four months under uniform laboratory conditions consisted of 337 males and 311 females. In this population, blues were more numerous than either of the other phenotypes as expected, but they fell about 40 short of the expected 50 percent of the entire population, which perhaps is an indication of inferior survival value of blue zygotes. There was practically no mortality subsequent to weaning. Blues weighed less than grays or cinnamons at four months of age in both sexes. Blue females averaged about 10 grams less in weight than gray females, and blue males averaged 33 grams less than gray males. The differences are highly significant, exceeding ten times the probable error in the case of males and five times in the case of females.

We must conclude therefore that the blue mutation is on the whole disadvantageous. Blues probably have less survival value than non-blues of identical parentage, and those which do survive are of significantly smaller body size than their non-blue litter mates.

The case is very different with cinnamons. What their relative survival value is remains uncertain because the genotype of the blue parent was not ascertained for the individual matings. The average body weight of the cinnamons is in both sexes greater than that of their gray litter mates, but the difference is too small to have statistical significance, being less than the probable error in the case of males and less than twice the probable error in the case of females.

If we compare the body weight of blues with that of their non-blue litter mates whether cinnamon or gray, we find the average weight of 196 non-blue males to be 367.0 ± 2.1 grams, whereas that of 141 blue males is 333.2 ± 2.1 grams. The difference between these average values is 33.8 ± 2.9 grams, or more than 11 times the probable error. Comparing the body weight of blue females with that of non-blue (gray or cinnamon) females, we find the average difference to be 24.2 ± 2.8 grams, a difference nearly nine times its probable error, and so highly significant.

It will be observed that cinnamons were slightly heavier than their gray litter mates in both sexes. Though the differences are small and not statistically significant they are in agreement with those found between similar color varieties in mice. Additional support comes from some observations made of backcross litters which were not raised to the age of four months but were destroyed at weaning time, age about one month. Sixteen gray males averaged 99.5 grams in weight; eight cinnamon males averaged 106.7 grams, and 16 blue males averaged 93.0 grams.

The females in these same litters averaged as follows: 17 gray females, averaged 90.2 grams; three cinnamon females averaged 98.6 grams; 25 blue females averaged 79.0 grams.

The relative body weights are the same as in the case of the animals weighed at an age of four months—namely, cinnamons largest, grays next, and blues least. Since the numbers of individuals in this group are small and their probable errors large, the differences between the color groups are not of sufficient magnitude to be statistically significant except in the case of blue females as compared to non-blue females, where the difference in the means exceeds three times the probable error of the difference. Nevertheless, considerable weight attaches to the qualitative uniformity of the size differences. In both sexes, at one month of age as well as three months later, cinnamon individuals as a group are heavier than grays, and both are heavier than blues. Consequently we may conclude that in rats as well as in mice the brown (cinnamon) mutation probably adds to

body size. But in rats the blue mutation certainly decreases body size whereas in mice the blue mutation increases body size. It may be that what we call blue in rats is not the equivalent of what we call blue in mice but possibly of some similar phenotype such as leaden in mice, which decreases instead of increases body size, although phenotypically it is indistinguishable from blue.

INFLUENCE OF THE BROWN MUTATION ON BODY SIZE IN RABBITS

In connection with a study of size inheritance in rabbits the question was several years ago thoroughly investigated whether or not the blue mutation or genes associated with it in the same chromosome exert any appreciable influence on body size. No such influence could be detected. Accordingly we must conclude that the blue mutation in rabbits, unlike either the blue or the leaden mutations of mice or the blue mutation of rats, has neither an accelerating nor a retarding influence on growth and body size. In the same investigation of size inheritance in rabbits, it was also shown that the mutations non-agouti, English, and yellow, as well as blue, are without influence on body size. It remained, however, to discover whether the brown mutation in rabbits has or has not accelerating effects such as it has in both mice and rats. For this purpose a cross was made between a male rabbit of a small race having dilute sooty yellow pigmentation and chocolate brown eyes, its genetic formula being *aa bb CC dd ee*, and females of a large New Zealand Red race, whose genetic formula is *AA BB CC DD ee*. The F_1 animals were of intermediate size and in color resembled their large-sized mothers, their formula being *Aa Bb CC Dd ee*. F_1 females were now back-crossed to their father or to another male of like genetic constitution. Segregation occurred for genes *Aa* and *Dd*, but since it has been abundantly demonstrated that the *a* and *d* mutations in rabbits do not affect body size, we may disregard these and focus our attention on the segregation as regards the *Bb* alleles.

The back-cross rabbits were weaned at the age of one month and kept under uniform conditions until they were four months old. At this age rabbits, like rats, are not fully grown, but growth is sufficiently advanced to show what the relative sizes will be when growth has been completed.

The back-cross population reared to this age consisted of 106 rabbits which may be classified as follows: 16 black pigmented males averaged 1717 ± 20 grams; 30 brown pigmented males averaged 1758 ± 24 grams; 18 black pigmented females averaged 1739 ± 31 grams; 32 brown pigmented females averaged 1778 ± 25 grams.

Since female rabbits are little heavier than males at four months of age, we may properly combine the data for both sexes, which gives us this

result. The black pigmented individuals total 34, average weight 1728 ± 22; brown pigmented individuals total 62, average weight 1768 ± 17 grams. Brown individuals are on the average 40 ± 28 grams heavier than black ones. This is not a statistically significant difference, since the numbers are small, but for the following reason probably indicates a genuine genetic trend. Although the brown pigmented ancestor is of much smaller body size than the black pigmented one, brown individuals average heavier than black ones in the backcross population. This is true in both sexes and is consistent with the physiological action of brown observed in numerous mouse crosses and in the cross with rats already described.

It would be of interest to know whether or not a brown mutation has a similar relation to body size in dogs, horses, guinea pigs and Peromyscus among mammals and in the canary among birds, in all of which species a brown mutation has been shown to be recessive in inheritance.

SUMMARY

In a back-cross population of 608 mice, in which segregation occurred simultaneously for the mutations leaden and brown, it was found that leaden individuals are significantly smaller than non-leaden in weight, body length and tail length.

Among the non-leaden individuals, all of which are heterozygous for leaden, brown pigmented are slightly larger bodied than black pigmented ones, as is usual in other mouse crosses involving segregation for black versus brown. But the differences are so small that it is clear that leaden even when heterozygous diminishes the usual advantage of browns over blacks. When leaden is homozygous, the advantage of brown over black is reversed, and blacks are larger bodied than their brown sibs.

In a back-cross population of 2030 mice, in which segregation occurred simultaneously for the mutations pink-eye 2 and brown, it was found that pink-eyed individuals are significantly smaller than non-pink-eyed in weight, body length and tail length. The interaction between *p2* and *b* shows a retarding influence on growth, when either gene is heterozygous and the other homozygous, but when both are homozygous, the retarding influence is greater still.

The mutation *p2* is more strongly injurious than *l* both by itself and in its interaction with *b*.

The dominant gene A^y, which is lethal when homozygous, increases body size when heterozygous, the effect being stronger in females than in males.

The brown mutation increases body size in the Norway rat and the domestic rabbit, as well as in mice.

The "blue" mutation of the rat reduces body size and so has a physio-

logical action more like that of the leaden mutation than of the blue mutation of mice.

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GENETIC AND NON-GENETIC FACTORS WHICH INFLUENCE THE TYPE OF THE SKELETON IN AN INBRED STRAIN OF MICE[1]

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INTRODUCTION

STRUCTURAL and numerical variations in vertebrate skeletons are well known to anatomists. The physiological causes of such variations are little understood, although in a few instances genetic influences have been suggested. The relative importance of both genetic and non-genetic factors acting during the development of the skeleton is practically unknown. This paper is an attempt to evaluate such factors acting upon variations at the thoraco-lumbar and the lumbo-sacral borders of the vertebral column in the Bagg albino strain of the mouse, *Mus musculus*.

Many species of mammals have been sampled sufficiently to give an estimate of numerical variations in their vertebral columns. Typically there is a certain modal or normal class for each species, with variation about the mode. Characteristic breed differences have been discovered among swine by SHAW (1929), MOSKOFF (1934), and FREEMAN (1939), among horses by MOSKOFF (1934), and among rabbits by SAWIN (1937). Among the primates, the purpose of the work has largely been to discern phylogenetic relationships between species rather than elementary genetic relationships within a species. This study is concerned with variation within a species and is no contribution to phylogenetic problems, which have been adequately reviewed by FREY (1929), DANFORTH (1930), KÜHNE (1931, 1936), and others.

Previous work on vertebral variations done from a genetic viewpoint has led to interesting, though discordant, hypotheses. PROMPTOFF (1928) examined 4000 individuals in three races of domestic poultry and their crossbred progeny for variations in the structure of the dorsosacrum. He was able to establish the fact that different types of pelves are hereditarily transmissible. The factorial explanation, which he advanced, based upon three pairs of alleles, seems best regarded as tentative until critical breeding tests are made. SHAW (1929) and TUFF and BERGE (1936) established

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an hereditary basis for variations in number of pairs of ribs and of presacral vertebrae in swine. Both pieces of work point to some variability which is not easily explained on simple genetic grounds. TUFF and BERGE found that, with respect to the number of presacral vertebrae, matings of 27×27, 28×28, and 29×29 each produced young with 27, 28, and 29 vertebrae, although in each case the parental type occurred most frequently in the offspring.

TABLE 1

Distribution in percentage of skeletal types in samples of eleven stocks of inbred mice. Thoracic to lumbar ratios are given under each class of presacral vertebrae.

STRAIN*	GENERATIONS INBRED	PRESACRAL VERTEBRAE									TOTAL
		25		ASYM.	26			ASYM.	27		
		12/6	13/5		12/7	13/6	14/5		13/7	14/6	
P	10	88.1		8.4	3.6						84
Gates	?	2.5		1.9	80.3	15.3					157
C_3H	40		96.5	2.5		1.0					200
dba	30		95.3	2.9		1.8					171
C	8		58.2	7.2		34.6					321
C_{57}Blk	20		2.1	2.5		95.4					241
Leaden	40		1.1	2.9		95.7	0.3				278
N	5		0.6	2.6		96.8					157
A	50		2.1	4.2		93.7					48
L	5					11.3	59.0	11.7		18.0	824
Bagg alb.	40					17.8	56.0	13.5	0.2	12.5	3026

* The author is indebted to the director and members of the staff of the ROSCOE B. JACKSON MEMORIAL LABORATORY for providing samples of the dba, C_{57}Blk, and A strains; to the same laboratory for parent animals of the P, Leaden, and Bagg albino strains; to DR. L. C. STRONG of YALE UNIVERSITY for parents of the C_3H strain; to DR. W. H. GATES of LOUISIANA STATE UNIVERSITY for parents of the Gates short-ear stock; and to MR. A. G. HUMES for permission to use the preliminary data which he gathered on the C, N, L, and Leaden strains.

On the basis of a study upon 123 human families and 106 pairs of twins, both mono- and dizygotic, KÜHNE (1931, 1936) formulated a single factor interpretation for vertebral variations. A variation in which a vertebra tends to assume the characteristics of the next anterior vertebra is termed a "caudal" tendency because the effect is to place the critical border at a greater distance from the cranium. A variation in which a vertebra tends to assume the characteristics of the next posterior vertebra, a "cranial" tendency, reduces the distance between the cranium and the critical border. "Cranial" is dominant to "caudal," according to KÜHNE's interpretation.

SAWIN (1937) and GREEN (1939) found that for the rabbit the presence of an extra pair of ribs (a "caudal" tendency) could not be explained on

the basis of a mendelian recessive factor, although certain proportions in hybrid generations simulated single factor segregation. Other generations gave ratios of normal and extra-ribbed individuals that could be explained neither by a single factor nor by any obvious multiple-factor hypothesis.

Clearly much more information from genetically controlled breeding in many species will be necessary to resolve the complexity of factors causing skeletal variations.

SURVEY OF INBRED STOCKS OF MICE

With a view toward obtaining the preliminary information necessary for a genetically controlled breeding scheme, eleven laboratory stocks of the house mouse, six long inbred and five recently inbred (brother × sister), were examined for their skeletal compositions. All animals were prepared for recording by a routine potash clearing and alizarin staining technique, such as that described by CUMLEY et al. (1939). The results of the survey are given in table 1, and different skeletal types are shown in figure 1.

Two types of problems emerge from this information: (1) what are the causes of variation within the highly inbred strains, and (2) what are the causes of the differences between inbred strains? Variations of the first sort only are considered here. For this purpose the Bagg albino stock was chosen because it has variations in number of ribs and position of sacrum occurring with such frequency that analysis is reasonably practicable. Further, the Bagg stock has desirable characteristics of vigor, large and frequent litters, low incidence of mammary carcinoma, and high resistance to common laboratory diseases.

THE BAGG ALBINO STRAIN OF MICE

The 3026 individuals of this strain examined for skeletal type were descended from a single pair of the 36th generation of inbreeding. The stock was continued by matings of brother and sister, usually litter-mates, and may be divided arbitrarily into five sublines, each subline having descended from single matings in the 38th or 39th generation. Only individuals produced after the 38th or 39th generation and up to the 43rd generation have been included in this summary. The distributions of the various skeletal compositions within sublines are shown in table 2. The formulae, 13/6, 14/5, etc., refer to the numbers of thoracic and lumbar vertebrae. In cases of asymmetry in the position of the innominate bones articulating with the sacrum, the count of lumbar vertebrae on the right side is given above, the count on the left side below, as $13/\frac{1}{7}$. In a low percentage of animals, there is a slight tendency, distinct from asymmetries, for the last lumbar vertebra to transform into a sacral vertebra. This is

detectable chiefly as a different contour of the traverse processes. Because these finer gradations are low in frequency and difficult to classify, the

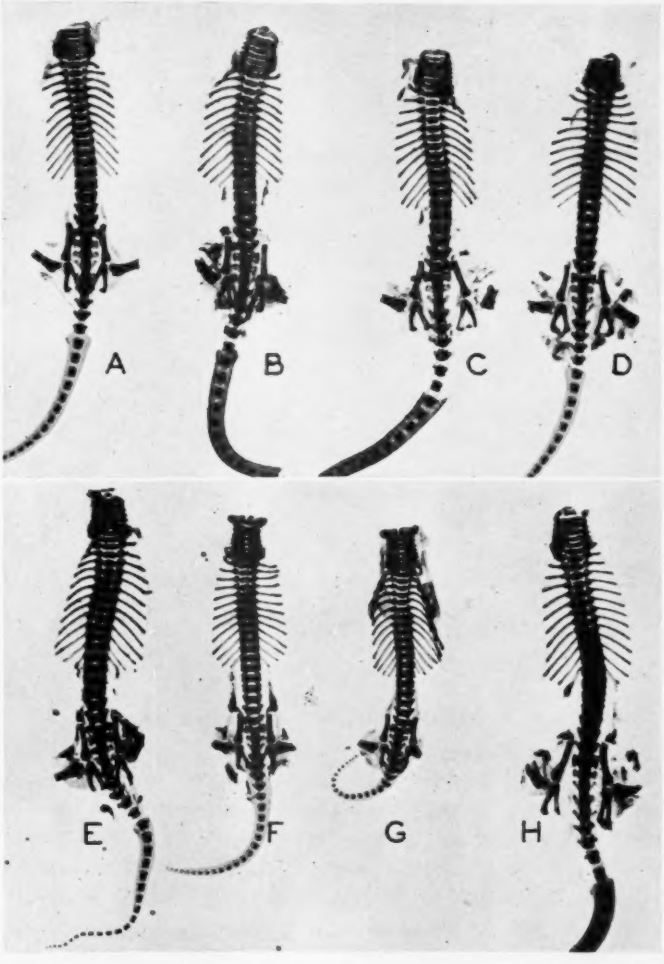

FIGURE 1.—Ventral aspects of skeletons of young mice with different ratios of thoracic to lumbar vertebrae. A, 13/5; B, 13/$\frac{5}{6}$; C, 13/$\frac{6}{5}$; D, 13/6; E, 14/5; F, 14/$\frac{5}{6}$; G, 14/$\frac{6}{5}$; H, 14/6.

most objective procedure for purposes of analysis seems to be to classify them with the type which they most closely resemble. Thus in the descriptions, "26-psv" will be taken to mean the group of individuals having 26

or nearly 26 presacral vertebrae, and "27-psv" will mean those not having 26 presacral vertebrae, except when the asymmetrical types are specifically excluded. Nor is a detailed classification made in table 2 for degree of development and for asymmetry of the ribs. An individual having any evidence of a 14th rib, whether on one side or both, is classed as 14-ribbed. Only litters larger than one in which all or almost all the members were recorded for skeletal type were used in the computations.

TABLE 2

Distribution of vertebral types in sublines of Bagg albino strain of mice.

SUBL.	PRESACRAL VERTEBRAE AND THORACO-LUMBAR RATIOS								TOTAL
	26		R26/L27		R27/L26		27		
	13/6	14/5	13/$\frac{6}{7}$	14/$\frac{5}{6}$	13/$\frac{7}{6}$	14/$\frac{6}{5}$	13/7	14/6	
1	96	242	4	11	0	29	0	43	425
2	97	344	1	35	5	53	2	92	629
3	86	336	1	26	3	59	2	74	587
4	148	479	2	42	3	69	1	107	851
5	112	294	1	23	0	42	0	62	534
Total	539	1695	9	137	11	252	5	378	3026

Summary:	TYPE	NUMBER		PERCENT	
	13-ribbed	564		18.64	
	14-ribbed	2462		81.36	
	26-psv*	2234		73.83	
	Asymmetrical	409	792	13.52	26.17
	27-psv	383		12.65	

* psv = presacral vertebrae.

This observable variation in the Bagg albino may be considered as involving two vertebral borders: (1) The lumbo-sacral border—variation in the position of the sacrum, depending upon whether there are 26 or 27 presacral vertebrae. (2) The thoraco-lumbar border—variation in the number of pairs and degree of expression of ribs. The two borders will be treated separately in the analysis, but their interdependence will not be overlooked.

VARIABILITY IN NUMBER OF PRESACRAL VERTEBRAE

Genetic factors

For the purpose of estimating the effect of genetic factors on the variation in the number of presacral vertebrae, the population may be subdivided in at least three ways—(1) by sex, (2) by subline, and (3) by phenotypes of mated parents.

Sex and number of presacral vertebrae

A system of continued brother-sister mating automatically results in homozygosis for autosomal factors and identity of X chromosomes. But mated individuals will differ in their "skeletal" factors, if such factors are located on the sex chromosomes, quantitatively if on the X chromosome, perhaps qualitatively if on the Y chromosome. It is important, therefore, to examine the population for a possible association between sex and number of presacral vertebrae. Table 3 shows by sex the frequency of individuals with 26 and 27-psv in the five sublines.

TABLE 3

Distribution of presacral vertebrae by sexes in sublines of Bagg albino strain of mice.

SUBL.	MATINGS	LITTERS	26-psv			27-psv			TOTAL			PORTION 26-psv
			♂♂	♀♀	♂♀	♂♂	♀♀	♂♀	♂♂	♀♀	♂♀	
			A	B	C	D	E	F	G	H	I	
1	17	62	177	161	338	26	61	87	203	222	425	.795
2	23	103	246	195	441	66	122	188	312	317	629	.701
3	25	82	234	188	422	39	126	165	273	314	587	.720
4	30	128	369	258	627	73	151	224	442	409	851	.737
5	26	96	225	181	406	36	92	128	261	273	534	.760
Total	121	471	1251	983	2234	240	552	792	1491	1535	3026	.738

Homogeneity tests for:	COLUMNS	χ^2	DF	P
1. Sex distribution in sublines	G, H	4.8	4	30%
2. Sex distribution in psv-classes in total	Sums of A, B, D, E	154.7	1	<1%
3. Sex distribution in psv-classes of sublines, on basis of sex distribution in total	A, B	3.9	4	40%
	D, E	6.4	4	15%
4. Distribution of presacral vertebrae in sublines, no sex distinction	C, F	13.8	4	1%
5. Distribution of presacral vertebrae in males of sublines	A, D	9.5	4	5%
6. Distribution of presacral vertebrae in females of sublines	B, E	10.8	4	3%

In the total population there are 1491 males and 1535 females, and if there were no association between sex and vertebral type, approximately equal frequencies of the two sexes might be expected in the 26 and 27-psv classes. An examination of the sex distribution in the total population and in the sublines shows a definite and uniform bias toward males with 26 presacral vertebrae and toward females with 27 presacral vertebrae. The χ^2 test based on the totals indicates a probability of much less than 1 percent of obtaining such a sex distribution by random sampling ($\chi^2 = 154.7$, degrees of freedom, DF = 1).

The effect of sex upon the vertebral type can be determined by computing the portion of the total variance associated with sex. Here, however, we encounter the difficulty that the variable character, the number of presacral vertebrae, occurs not in a normally graded series, but practically in alternative categories. It seems reasonable to assume that back of this dichotomy there is a continuous scale of factor combinations, approximately normally distributed, such that values which fall on one side of a certain physiological threshold produce 26 presacral vertebrae and values which fall on the other side produce 27 presacral vertebrae or asymmetrical types. This concept has been used previously by PEARSON (1901) for the correlation surface represented by a four-fold table, by WRIGHT (1920, 1934) for spotting and polydactyly in the guinea pig, and by FISHER (1930) for vertebrae.

If we were dealing with a continuous variable, it could be shown that the variance in the total population (σ_t^2) is equal to the sum of two separate variances, that within the sexes (σ_w^2) and that between the sex means (σ_m^2), $\sigma_t^2 = \sigma_w^2 + \sigma_m^2$.

Because vertebrae occur in whole numbers and there is no convenient gradation between 26 and 27-psv, none of the variances can be computed directly. For each sex, however, the distances (x) between each sex mean and the threshold separating 26 and 27-psv can be computed in units of the standard deviation, from the relationships, $x = \text{prf}^{-1}(p - \frac{1}{2})$, the inverse probability function. Simply, this means that if one knows the area $(p - \frac{1}{2})$, the deviate (x) to which this area corresponds can be found from a table of deviates in terms of areas of the normal curve. In this symbolism p represents the proportion of 26-psv in each sex. This assumes that $\sigma_w^2 = 1$. The variance of the x-distances (σ_x^2) is equal to the square of one-half the difference between the male and females distances, $\sigma_x^2 = ((x_{\sigma^*} - x_{\varphi})/2)^2$.

The variance between sex means (σ_m^2) is equal to the product of the within sex variance (σ_w^2) and the variance of the differences between the sex means and the vertebra threshold (σ_x^2), $\sigma_m^2 = \sigma_w^2 \cdot \sigma_x^2 = \sigma_x^2$. We are interested in knowing what portion of the total variance (σ_t^2) is due to variation between sexes. The desired ratio is:

$$\sigma_m^2/\sigma_t^2 = \sigma_x^2/1 + \sigma_x^2.$$

This gives the ratio in terms of computable values. According to WRIGHT (1934), who devised this method, the result given by it is not essentially different from that given by the squared biserial eta method. By performing such a computation for the effect of sex on presacral vertebrae, it was found that 0.09 or 9 percent of the total variance is due to sex. This relationship is similar to that found by SAWIN (1937) for an extra presacral vertebra in the rabbit.

Distribution of presacral vertebrae in sublines

If for any reason (recent mutation, failure of genetic fixation) the original parents of the 36th generation were unlike in genotype, other than for the sex factors, then the separation of their progeny into non-interbreeding sublines should eventually cause segregation and fixation of the genetic factors by which they differed. Highly significant subline differentiation would signify that real genetic differences distinguished the two parents or that new and different mutations were accumulating in the sublines. Before testing for subline differentiation, three possible sources of error should be noted. (1) The sublines may not be separated from their common origin by a sufficient number of generations to make differentiation detectable. At the present stage of the experiment this circumstance cannot be dismissed on theoretical grounds, but it appears to introduce no great error, because the estimate of genetic differentiation based on the sublines agrees with that based on the parent-offspring correlation (next subsection). (2) In view of the known association between sex and vertebral type, an unusual sex distribution within a subline could be mistaken for genetic differentiation. This does not seem serious, however, because there is no unexpected variation in the frequency of sexes, and $\chi^2 = 4.8$ for four degrees of freedom is well within the limits of expectation. (3) Another circumstance which might be mistaken for genetic differentiation is resemblance of litter mates caused by the action of non-genetic factors during uterine development. It will be shown later that such factors are present, although they account for less than 1/10 of the total variance.

Of the entire population, 73.8 percent had 26 presacral vertebrae. The last column of table 3 gives the proportion of this type in each subline, no distinction for sex being made. The test for homogeneity based upon 0.738 as the portion which should characterize each subline, gives a probability of slightly less than 1 percent. When retested, using only the males in each subline and 0.839 (1251/1491) as the characteristic proportion, the probability exceeds only the 5 percent level. The same occurs when the females alone are used for retesting, the proportion expected being 0.640 (983/1535). Thus it appears that the substrains are different, but by such a small amount that non-genetic factors acting similarly on litter mates may account for the apparent differentiation.

If there is subline differentiation, then the portion of the total variance of the population due to subline differentiation will give an estimate of the extent to which genetic factors contribute to the total variance. The method of computation is similar to that used in the preceding section except that the variance of the distances (σ_x^2) between the vertebra threshold and the subline means is found by properly weighting for each subline the squared deviations of the x-values from their mean, $\sigma_x^2 =$

$1/NSn(x-\bar{x})^2$, where $\bar{x}$ $(=1/NSnx)$ is the weighted mean of the x-values, where n represents the number of individuals in successive sublines, and where N is the total number in the population.

By using the formula given previously, $\sigma_x^2/(1+\sigma_x^2)$, it was found that 0.01 or about 1 percent of the total variance could be accounted for on the basis of genetic differences between the original parents.

This value is subject to errors introduced by assuming normality of the distribution of unknown factors within the sublines, by assuming the equality of the variances within the sublines, and by basing the estimate upon the totals for each subline with no distinction for sex. Since none of these sources of error appears to have more than slight weight, the calculated value is accepted as showing the general magnitude of the contribution of genetic factors other than those associated with sex to the total variance.

Parent-offspring correlation

Genetic factors causing variation in the position of the sacrum can also be detected by classifying the offspring according to the phenotypes of the parents (table 4). In the total population, 73.9 percent of the animals are 26-psv, and this percentage rather closely characterizes the progeny from each type of mating, as shown by the χ^2 test. There is thus no evidence

TABLE 4

Distribution of presacral vertebrae in young of different types of matings, all sublines.

MATING ♂♂ ♀♀	LITTERS	NUMBER	PORTION 26-psv
26×26	268	1706	.748
26×27	93	564	.743
27×26	74	495	.701
27×27	29	209	.742
Total	464*	2974*	.739

$\chi^2=3.9$ DF=3 P=30%

Average, parent-offspring, r=0.03

* Because of unclassified parents, 52 mice in 7 litters are omitted.

for a significant correlation between parent and offspring, the average of which is 0.03. The tetrachoric coefficients of correlation here used have been computed from diagrams prepared by CHESIRE, SAFFIR, and THURSTONE (1933).

Both the non-significance of this correlation and the estimate of portion of total variance due to subline differentiation may be taken to signify that genetic factors, other than those associated with sex, are practically

negligible in determining the variability in the position of the sacrum of Bagg albino mice.

Non-genetic factors

In order to detect and evaluate possible non-genetic agents acting upon the position of the sacrum, the population may be considered as being composed of (1) sibships, those individuals produced by single pairs of parents, (2) litters, and (3) individuals. Since individuals are frequently (13.5 percent) asymmetrical, opposite sides may be considered independently.

Sibship correlation

A table for estimating the sibship correlation may be constructed by correlating the individuals of a given litter, not with one another, but with the individuals in all other litters produced by the same parents.

The presence of a significant sibship correlation could be caused by genetic similarity within sibships and genetic dissimilarity between sibships, or by long continued conditions of the mother's uterus, independent of her genotype and possibly a reflection of her physiological state. The first possibility may be disregarded in this case because genetic differences were practically nonexistent. Since a sibship correlation, $r = 0.01$, is practically absent, non-genetic influences acting upon whole sibships may also be considered as negligible.

Litter mate correlations

Tendencies toward resemblance of litter mates could be brought about by genetic and by non-genetic factors acting during prenatal development upon the litter as the unit. In this case we cannot easily exclude the factors associated with genotype. The autosomal factors may still be disregarded, but allowance must be made for the correlation between sex and number of presacral vertebrae. If there is an unusual distribution of sexes within litters so that excessive numbers of litters are predominantly male and others female, the distribution of numbers of presacral vertebrae within those litters will also tend toward abnormality. Ideally each size litter should be tested for the distribution of number of presacral vertebrae within it, according to the number of males in the litter. Unfortunately the total number of litters examined was not great enough to make such a tabulation meaningful. We can, however, determine whether or not the distribution of sexes within litters of all sizes is reasonably close to expectation on a random basis.

For the 24 litters of size 2, litters composed of 0, 1, or 2 males occur in the frequency 6, 15, 3. Litters of this sort were expected in the frequency 6, 12, 6, or the successive terms of $24(\frac{1}{2}+\frac{1}{2})^2$, where $\frac{1}{2}$ to $\frac{1}{2}$ is the expected

sex ratio. Similarly the expectations for the litters of size 3 are given by the expansion of $33(\frac{1}{2}+\frac{1}{2})^3$, and so on for all litter sizes. The tests for significance (χ^2) show that in no size of litter is the distribution of sexes sufficiently aberrant to be called non-random. Therefore it seems that the distribution of sexes by itself does not greatly distort the distribution of vertebral types within litters.

Table 5 gives the occurrence of animals of 26-psv type in litters of all sizes. The expected frequencies, as with the sex distribution, are computed by expanding a binomial with the appropriate ratio of portion 27-psv to portion 26-psv in each litter size. Where the frequencies of terminal classes are low, the classes are grouped. The degrees of freedom associated with each χ^2 test are two less than the number of classes, since both the total number and proportion of vertebral types within each size of litter are accepted in the calculations. If there are appreciable non-genetic factors acting upon the individuals which are gestated simultaneously, the litter mates should tend to resemble one another. Thus the frequencies in the terminal (unmixed) classes in each array should exceed the expected, and the frequencies in the middle (mixed) classes should be less than expected. An inspection of the directions of the deviations (D) of observed (O) from expected (E) in all sizes of litters shows that in general unmixed litters do occur more often and mixed litters less often than expected. The only noteworthy exception to this occurs in litters of size 5.

When these deviations are examined for significance, in only one case (size 9) do the deviations have sufficient magnitude to be highly improbable on the basis of a random distribution of the 26-psv type. The conformity of the distribution in the other litter sizes toward deviations in the same direction should not be overlooked. This relatively slight tendency toward concurrence of the litter mates may be caused wholly or in part by non-genetic factors, or by the distribution of sexes, although the sex distribution is random in the litters as a whole. Since it is not possible to resolve this complexity with the information now on hand, we shall regard the deviations as evidence for non-genetic influences acting on litter mates and attempt to evaluate their effect.

Correlations between litter mates are estimated from 2×2 tables made up for litters of size 3 to 10 (table 6). Such tables are constructed by correlating each member of a litter with all other members taken one at a time. The standard errors are computed according to Pearson's formula for the tetrachoric correlation coefficient, multiplied by a correction term to allow for the repetition of individuals in forming the table. This term is $\sqrt{[1+(n-1)r]/(1+r)}$, where n is the litter size. The correlations are averaged by the formula, $S(r/\sigma_r^2)/S(1/\sigma_r^2) \pm 1/S(1/\sigma_r^2)$ (WRIGHT 1934). From the average, it appears that eight percent of the total scale of ver-

TABLE 5

Distribution of mice with 26 presacral vertebrae in litters of all sizes. O = observed, E = expected, D = deviation.

LITTER SIZE		NUMBER WITH 26 PSV IN LITTER												LITTERS	26 PSV		χ^2	DF	P
		0	1	2	3	4	5	6	7	8	9	10	11		NUMBER	PORTION			
2	O	4	10	10	—	—	—	—	—	—	—	—	—	24	30	.625	0.7	1	40%
	E	3.4	11.2	9.4															
	D	+	−	+															
3	O	3	8	11	11	—	—	—	—	—	—	—	—	33	63	.636	1.7	1	20%
	E		9.9	14.6	8.5														
	D		+	−	+														
4	O	0	6	4	17	16	—	—	—	—	—	—	—	43	129	.750	0.6	1	40%
	E			11.3	18.1	13.6													
	D			−	−	+													
5	O	0	2	6	15	29	9	—	—	—	—	—	—	61	220	.721	2.7	2	25%
	E			8.3	17.8	23.0	11.9												
	D			−	−	+	−												
6	O	0	0	8	7	18	25	8	—	—	—	—	—	66	282	.712	1.2	2	45%
	E				14.8	21.0	21.2	9.0											
	D				+	−	+	−											
7	O	0	1	3	9	10	25	19	10	—	—	—	—	77	383	.711	6.1	3	10%
	E				8.7	16.7	24.5	20.1	7.0										
	D				+	−	+	−	+										
8	O	0	0	0	2	7	17	19	22	16	—	—	—	83	515	.776	5.6	3	15%
	E				6.7		14.7	25.5	25.2	10.9									
	D				+		+	−	−	+									
9	O	0	1	0	2	2	8	9	5	11	10	—	—	48	322	.745	12.6	2	<1%
	E				8.5			11.4	14.3		13.8								
	D				+			−	−		+								
10	O	0	0	0	0	0	2	2	8	4	5	6	—	27	215	.796	3.3	1	7%
	E					8.9				8.2	9.9								
	D					+				−	+								
11	O	0	0	0	0	0	1	1	0	2	0	2	1	7	58	.753	—	—	—
12	O	0	0	0	0	0	0	1	0	0	0	0	1	2	17	.708	—	—	—
											Total			471	2234	.738			

tebra determining factors are non-genetic influences acting upon litter mates, and the magnitude of the standard error supports the significance of this figure.

This correlation considers the individuals as units, but since the two sides of the same individual are frequently different, it is interesting to estimate the correlation between the same sides of litter mates. The tabulations are in table 7. The right sides of litter mates are determined by common factors to the extent of 10.6 percent of the total scale and left sides by 8.5 percent. Thus about 9.5 percent of the total scale of factors act similarly upon the sides of litter mates. (In order to keep a constant ratio between the sex effect and the litter mate concurrence when consideration is transferred from individuals to single sides, the effect of sex is now assumed to be 10.5 percent of the total variance.)

TABLE 6

Tetrachoric correlations between litter mates for litters of size 3 to 10, presacral vertebrae.

LITTER SIZE	LITTERS	psv	26	27	r	σ_r
3	33	27	19	17	.26	.07
		26	44	19		
4	43	27	42	22	.20	.05
		26	151	43		
5	61	27	124	48	−.01	.02
		26	314	124		
6	66	27	195	87	.05	.02
		26	512	196		
7	77	27	314	154	.09	.02
		26	835	314		
8	83	27	387	137	.10	.02
		26	1413.	387		
9	48	27	282	158	.22	.03
		26	1006	282		
10	27	27	187	60	.08	.03
		26	780	188		

Average, litter mate, $r=0.08\pm0.01$

It is interesting to examine the records for evidence of tangible non-genetic factors which may account for this resemblance either wholly or in part. Contributory circumstances may be (1) age of mother, (2) size

of litter, (3) season of year. Since amount of feeding, amount of light, temperature, gestation period, body weight and length, etc., were not recorded no test for their association with vertebral type can be made. From TUFF and BERGE'S (1936) records on pigs, it might be expected that extra vertebrae are associated with longer and heavier bodies.

(1) *Age of mother.*—Female mice have their first litters during the third or fourth month of age usually, and in this experiment a few were allowed to breed past the twelfth month. The frequencies of 26 and 27-psv types

TABLE 7

Tetrachoric correlations between single sides of litter mates for litters of size 5 to 9, presacral vertebrae.

LITTER SIZE	psv	RIGHT-RIGHT				LEFT-LEFT			
		26	27	r	σ_r	26	27	r	σ_r
5	27	97	31	.07	.03	102	22	.08	.03
	26	385	97			384	102		
6	27	179	68	.06	.02	136	36	.06	.02
	26	563	180			681	137		
7	27	266	107	.12	.02	223	82	.20	.03
	26	977	267			1089	223		
8	27	340	94	.09	.02	290	56	.05	.01
	26	1550	340			1687	291		
9	27	235	113	.27	.03	241	71	.11	.02
	26	1145	235			1175	241		
				0.106±0.010				0.085±0.009	
Average, single sides of litter mates, r=0.095									

in the progeny produced by 121 mothers from 3 to 12 or more months of age are given in table 8. The last column shows a suggestion of a gradual increase in the frequency of 26-psv as the mothers age. The χ^2 test made by assuming that 73.8 is the percentage of 26-psv which uniformly characterizes the progeny of each age group shows that there are very real differences between the groups. Because of rather marked fluctuations in the month to month percentages, it appears that some variation is associated with factors other than age of mother. If the age of mother were the most significant cause of variation, and if the relationship between age of mother and proportion of 26-psv were truly rectilinear, then the deviations from a regression line, fitted by least squares, should be reduced to non-significance. The χ^2 value is reduced, but the probability of obtaining such a deviation through random causes is still so small, though to be sure non-sig-

nificant, that it is impossible to say that the total effect is one of maternal age.

By using the method previously outlined for estimating the portion of the total variance due to variation between group means, it is found that 8.5 percent of the total variance is due to age of mother or that practically all the factors which act alike upon litter mates (8 percent) are in some way associated with maternal age. This figure is undoubtedly magnified by other unidentified causes. The effect is described in terms of maternal age, even though paternal age is identical or nearly so throughout the population.

TABLE 8

Distribution of presacral vertebrae by age of mother.

MOTHER'S AGE, MONTHS	LITTERS	NUMBER	PORTION 26-psv
3	49	252	.702
4	76	457	.705
5	68	483	.689
6	88	596	.762
7	65	437	.778
8	51	342	.731
9	29	216	.819
10	17	99	.737
11	12	61	.738
12	7	44	.750
13+	9	39	.769
Total	471	3026	.738

On assumption that 0.738 of total are 26-psv in each age group:
$\chi^2 = 22.7$ DF = 10 P = 1%
On assumption that the portion of 26-psv = .696 + .0059 (Age):
$\chi^2 = 16.6$ DF = 9 P = 6%

(2) *Size of litter.*—It may be thought that the sheer number of individuals being carried simultaneously in the mother's uterus may have a direct influence on the number of vertebrae through physical pressure during the formation and deposition of bone, or an indirect influence through the physiological competition of the litter mates for certain necessary substances available in limited quantity.

A direct tabulation of vertebral types by litter size would be somewhat misleading, because litter size tends to be a function of the age of the mother. Females tend to produce small litters when young, reach a reproductive maximum at six to eight months, and produce small litters again after 12 months.

Another variable possibly exerting an effect on the distribution in the vertebra classes and perhaps confounded with litter size is the frequency

of the mother's parity. In the data available it is not possible to separate independent effects of age of mother and of parity because of the high correlation between them, $r = 0.895 \pm 0.046$.

The best approach to the possible effect of size of litter seems to be through litters produced by mothers of the same age and of the same previous parity. Three examples are given in table 9, with suitable groupings of terminal litter classes to give larger numbers. In no case does the χ^2 test reveal any departures of observed from expected frequency of vertebral type greater than anticipated on the basis of random deviation within litters of all sizes. It seems reasonably clear, therefore, that litter

TABLE 9

Distribution of presacral vertebrae in litters of different sizes, with age of mother and parity constant. Group A: First litters from mothers 3 months old; 47 litters, mean size 5.2. Group B: Second litters from mothers 4 months old; 35 litters, mean size 6.7. Group C: Second litters from mothers 5 months old; 45 litters, mean size 7.4.

A			B			C		
LITTER SIZE	NUMBER	PORTION 26-psv	LITTER SIZE	NUMBER	PORTION 26-psv	LITTER SIZE	NUMBER	PORTION 26-psv
2–4	40	.725	2–5	41	.659	2–5	30	.733
5	60	.767	6	24	.702	6	42	.667
6	60	.667	7	28	.714	7	49	.776
7	42	.667	8	56	.750	8	96	.750
8–10	42	.714	9	45	.778	9	63	.698
			10–11	42	.714	10–11	52	.615
Total	244	.709	Total	236	.733	Total	332	.711
$\chi^2 = 1.8$ DF = 4 P = 80%			$\chi^2 = 2.2$ DF = 5 P = 80%			$\chi^2 = 4.5$ DF = 5 P = 50%		

size of itself has no influence on the variability in the position of the sacrum, neither directly in a mechanical manner nor indirectly in a physiological manner.

(3) *Season of year.*—Frequencies of the two principal types of vertebral columns may be classed by month of the year. While the χ^2 test shows that there is variation in the occurrence of 26 and 27 presacral vertebrae of such magnitude that the monthly proportions cannot be considered as homogeneous, still there is no obvious cycle in the proportions, and it seems that at present no conclusion can be drawn about the effect of time of birth upon the skeletal type.

Correlation between the sides of individuals

Since the two sides of individuals are not always identical with respect to the attachment of the innominate bones to sacral vertebrae, it is impor-

tant to evaluate the degree to which the two sides are determined by common factors. From table 10 it is found that 82 percent of the entire scale of factors causing variability in the number of presacral vertebrae act alike upon the two sides of individuals, whereas the remainder, 18 percent, act locally on single sides. The 82 percent includes the factors previously evaluated, those associated with sex, 10.5 percent, and those acting on litter mates, 9.5 percent. By subtraction, 62 percent of all the factors act similarly upon both sides of individuals independently of sex and of uterine environment.

TABLE 10

Correlation between sides of individuals, presacral vertebrae.

	psv	RIGHT		TOTAL
		26	27	
	27	146	383	529
Left	26	2234	263	2497
	Total	2380	646	3026

Between sides, r=0.82

Asymmetrical individuals comprise 13.5 percent of the total population. From table 10 it may be seen that the two asymmetrical classes, R26/L27 and R27/L26, occur in markedly different frequencies. When asymmetry occurs, the right innominate bone has the more posterior position about twice as frequently as does the left. The χ^2 method of testing (16.0, DF = 1, P < 1%) demonstrates that there is no doubt about the reality of this bias in the asymmetry. Its biological significance is uncertain. Although there is an abundance of information on systematic asymmetries of this sort in many mammals, there is a scarcity of unifying generalizations for their origins and causes.

VARIABILITY IN NUMBER AND EXPRESSION OF RIBS

Genetic factors

The steps in the analysis of variation in the number of pairs of ribs are comparable to those for variation in the number of presacral vertebrae.

Sex and the number of pairs of ribs

From the totals of table 11 it is possible to compute the portion of the total variance in number of pairs of ribs due to sex. This value is 0.05 or five percent. This compares with a similar association which MOSKOFF (1934) noted in the dog, horse, and cow, and which SAWIN (1937) found in the rabbit.

Subline differentiation

By use of the frequencies of table 11 and WRIGHT'S method for partitioning the variance, it is found that 1.2 percent of the total variance is accounted for by substrain differentiation.

TABLE 11

Distribution of ribs by sexes in sublines of Bagg albino mice.

SUBL.	MATINGS	LITTERS	13-RIB ♂♂	13-RIB ♀♀	13-RIB ♂♀	14-RIB ♂♂	14-RIB ♀♀	14-RIB ♂♀	TOTAL ♂♂	TOTAL ♀♀	TOTAL ♂♀	PORTION 13-RIB
			A	B	C	D	E	F	G	H	I	
1	17	62	65	35	100	138	187	325	203	222	425	.235
2	23	103	66	39	105	246	278	524	312	317	629	.167
3	25	82	55	37	92	218	277	495	273	314	587	.157
4	30	128	107	47	154	335	362	697	442	409	851	.181
5	26	96	76	37	113	185	236	421	261	273	534	.212
Total	121	471	369	195	564	1122	1340	2462	1491	1535	3026	.186

Homogeneity tests for:	COLUMNS	χ^2	DF	P
1. Sex distribution in rib classes in total	Sums of A, B, D, E	72.3	1	<1%
2. Sex distribution in rib classes of sublines, on basis of sex distribution in total	A, B	2.8	4	60%
	D, E	4.4	4	35%
3. Rib distribution in sublines, no sex distinction	C, F	14.0	4	1%
4. Rib distribution in males of sublines	A, D	13.8	4	1%
5. Rib distribution in females of sublines	B, E	2.9	4	60%

Parent-offspring correlation

If there is a measurable genetic determination involved in the number of pairs of ribs, the matings of different parental phenotypes should give

TABLE 12

Distribution of ribs in young of different types of matings, all sublines.

MATING ♂♂ ♀♀	LITTERS	NUMBER	PORTION 13-RIB
13×13	14	81	.185
13×14	84	557	.226
14×13	62	410	.181
14×14	304	1926	.179
Total	464*	2974*	.188

χ^2=6.4 DF=3 P=10%

Average, parent-offspring, r=0.05

* Because of unclassified parents 52 mice in 7 litters are omitted.

TABLE 13

Distribution of 13-ribbed animals in litters of all sizes. O = observed, E = expected, D = deviation.

LITTER SIZE		NUMBER WITH 13 RIBS IN LITTER											LITTERS	13-RIB NUMBER	13-RIB PORTION	χ^2	DF	P
		0	1	2	3	4	5	6	7	8	9	10						
2	O	17	7	0	—	—	—	—	—	—	—	—	24	7	.146	—	—	—
3	O	20	8	4	1	—	—	—	—	—	—	—	33	19	.192	3.0	1	10%
	E	17.4	12.4	3.2														
	D	+	−	+														
4	O	16	14	8	4	1	—	—	—	—	—	—	43	46	.267	2.0	1	15%
	E	12.4	18.1	12.5														
	D	+	−	+														
5	O	22	17	15	6	1	0	—	—	—	—	—	61	69	.226	4.9	2	10%
	E	16.9	24.7	14.5	4.9													
	D	+	−	+	+													
6	O	26	20	15	4	1	0	0	—	—	—	—	66	66	.167	2.7	2	25%
	E	22.1	26.5	13.3	4.1													
	D	+	−	+	+													
7	O	29	20	10	11	6	0	1	0	—	—	—	77	103	.191	22.6	2	1%
	E	17.5	28.8	20.4	10.3													
	D	+	−	−	+													
8	O	24	29	13	12	3	1	1	0	0	—	—	83	114	.172	7.6	2	2%
	E	18.4	30.5	22.1	12.0													
	D	+	−	−	+													
9	O	13	12	9	8	4	0	2	0	0	0	—	48	82	.190	7.9	2	2%
	E	7.2	15.3	14.3	11.2													
	D	+	−	−	+													
10	O	5	9	10	1	0	0	0	2	0	0	0	27	46	.170	—	—	—
11	O	0	5	2	0	0	0	0	0	0	0	0	7	9	.112	—	—	—
12	O	1	0	0	1	0	0	0	0	0	0	0	2	3	.125	—	—	—
									Total				471	564	.186			

markedly different progeny distributions. The results of the different types of matings are given in table 12, and it may be seen that the percentages of 13-ribbed individuals do not fluctuate markedly. Parent-offspring correlations average about 0.05. The average correlation has no statistical significance as shown by the χ^2 method.

The evidence from subline differentiation and parent-offspring correlation may be interpreted to mean that very little, if any, of the total variance is due to genetic factors other than sex, which alone accounts for five percent.

Non-genetic factors

Sibship correlation

The average of the sibship correlations is sufficiently close to zero that it may be disregarded.

Litter-mate correlations

The distributions of 13-ribbed mice within litters of all sizes are given in table 13. As with the distribution of vertebral types, the deviations of ob-

TABLE 14

Tetrachoric correlations between litter mates for litters of size 3 to 10, ribs.

LITTER SIZE	LITTERS	RIBS	13	14	r	σ_r
3	33	14	12	68	.39	.06
		13	7	12		
4	43	14	43	146	.29	.04
		13	26	43		
5	61	14	99	373	.13	.03
		13	39	99		
6	66	14	132	698	.03	.02
		13	28	132		
7	77	14	215	1093	.26	.02
		13	94	215		
8	83	14	307	1618	.15	.02
		13	92	307		
9	48	14	241	1159	.18	.02
		13	87	241		
10	27	14	152	856	.23	.02
		13	55	152		

Average, litter mate, $r=0.17\pm0.01$

served from expected are actually significant in only a few sizes of litters, 7, 8, and 9, but there are no important exceptions to the tendency for mixed litters to be deficient and unmixed litters excessive in number. SHAW (1929) observed this same tendency toward resemblance of litter mates for pairs of ribs in litters of pigs. The average litter-mate correlation, 0.17, estimated in table 14, includes not only the non-genetic factors, but also the genetic factors operating alike on litter mates. Since the residual genetic determination is negligible, no correction of this figure is made.

TABLE 15

Tetrachoric correlations between single sides of litter mates for litters of size 5 to 9, ribs.

LITTER SIZE	RIBS	RIGHT-RIGHT				LEFT-LEFT			
		13	14	r	σ_r	13	14	r	σ_r
5	14	132	274	.05	.03	118	284	.22	.03
	13	72	132			90	118		
6	14	184	539	.10	.02	184	543	.10	.02
	13	84	183			78	185		
7	14	293	868	.16	.02	305	832	.17	.02
	13	163	293			175	305		
8	14	421	1220	.20	.02	446	1241	.08	.02
	13	261	422			191	446		
9	14	298	954	.25	.02	345	871	.07	.02
	13	178	298			167	345		
				0.16±0.01				0.12±0.01	

Average, single sides of litter mates, r=0.14

Since there is also asymmetry in the occurrence of the 14th rib, the two sides of individuals may be considered separately. When same sides of litter mates are correlated (table 15), it is found that about 14 percent of the total variance is due to agents which act on single sides of litter mates. (The sex effect is proportionally reduced to 0.04.) The search for non-genetic factors associated with this resemblance revealed that, as with the number of presacral vertebrae, the age of the mother appears to be one of the contributory circumstances.

Individuals

Opposite sides of individuals resemble each other rather strongly, r=0.72 (table 16). The difference shows that 28 percent of the total scale of factors operate independently on single sides of individuals. The 72 per-

cent includes all the genetic factors and the non-genetic factors common to litter mates. When allowance is made for these, 54 percent of the factors are found to act on both sides of individuals independently of sex, of factors acting on whole litters, and of factors acting only on single sides.

TABLE 16

Correlation between sides of individuals, ribs.

	RIBS	RIGHT		TOTAL
		13	14	
	14	313	1834	2147
LEFT	13	564	315	879
	Total	877	2149	3026

Between sides, r=0.72

Expression of ribs

In the preceding discussion only the presence or absence of ribs was considered. We are interested now in examining further the degree of development of the ribs. All authors who have recorded variations in the thoraco-lumbar border in mammals have noted the frequent incomplete development of the ribs. The fourteenth rib of the mouse has been graded as 0, 1, 2, 3, 4 on each side, depending upon whether it is absent, 1/4, 2/4, 3/4, or 4/4 developed. Since these are visual estimates of rib expression, there are possibly frequent recording errors, but it is believed that their magnitude is not sufficiently great to invalidate the following general conclusions. A classification is made in table 17 for the frequency of males and females in all combinations of rib expression. A further subdivision is made for number of presacral vertebrae. From this information it is possible to make generalizations concerning (1) symmetry of rib development, (2) sex association with rib development, (3) sacralization and rib development.

Symmetry and rib development

By extracting the frequencies for symmetrical classes of rib development, 00, 11, . . . , 44, from the "totals" column of table 17, it is found that 1559 (766 ♂♂, 793 ♀♀) or 51.5 percent of the individuals are symmetrical. The relationship between the sides, 72 percent determination by common factors, previously found by means of the tetrachoric correlation coefficient, was based upon presence and absence only and it is not wholly adequate for expressing the symmetry of rib development.

Various grades of asymmetry occur, depending upon whether there are

0, 1, 2, 3, or 4 rib-units difference between the right and left sides. There are five symmetrical combinations, but the terminal classes, 00, 44, plus

TABLE 17

Distribution of males and females in all combinations of rib expression and presacral vertebrae.

RIBS		PRESACRAL VERTEBRAE								TOTALS	
		26		R26/L27		R27/L26		27			
R	L	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
	0	361	178	3	6	4	7	1	4	369	195
	1	28	17	2	1	0	1	0	0	30	19
0	2	43	22	0	1	2	2	1	0	46	25
	3	54	47	1	2	3	4	2	3	60	56
	4	27	35	2	5	2	3	0	3	31	46
	0	41	29	2	0	3	4	0	0	46	33
	1	49	21	0	1	0	0	0	2	49	24
1	2	18	13	1	0	1	0	1	1	21	14
	3	42	36	2	0	4	4	0	6	48	46
	4	22	30	2	2	4	8	3	6	31	46
	0	45	27	1	3	3	5	0	0	49	35
	1	19	5	0	0	0	1	1	0	20	6
2	2	23	24	1	1	1	1	0	4	25	30
	3	30	32	3	0	4	3	2	7	39	42
	4	15	22	1	1	0	3	2	5	18	31
	0	54	43	1	1	2	5	3	6	60	55
	1	37	20	1	2	1	4	0	3	39	29
3	2	21	20	1	7	1	4	3	4	26	35
	3	99	78	5	10	10	22	14	18	128	128
	4	50	47	7	12	2	16	11	25	70	100
	0	18	14	0	0	2	2	0	1	20	17
	1	17	12	0	1	3	4	1	6	21	23
4	2	8	9	0	1	1	3	1	2	10	15
	3	27	42	3	4	7	9	3	14	40	69
	4	103	160	15	31	24	64	53	161	195	416
Total		1251	983	54	92	84	179	102	281	1491	1535
Summary:											
13-rib		361	178			8	17			369	195
14-rib		890	805			232	535			1122	1340

33, occur with such high frequency that they are considered separately from the intermediate symmetrical classes, 11 and 22. These latter average 37.0 ♂♂ and 27.0 ♀♀ for each combination. There are eight

combinations of one rib-unit difference, averaging 36.5 ♂♂ and 39.7 ♀♀; six of two rib-units difference, averaging 35.0 ♂♂ and 30.2 ♀♀; four of three rib-units difference, averaging 43.0 ♂♂ and 45.0 ♀♀; and two of four rib-units difference, averaging 25.5 ♂♂ and 31.5 ♀♀. It is evident that if the terminal symmetrical classes are not considered, the frequencies of the other symmetrical and asymmetrical classes have the same general magnitude, and there is no obvious tendency for the ribs on opposite sides to develop to the same extent. The excess numbers in the terminal symmetrical classes are probably caused by biological limitations, there being both an absolute maximum (complete extra ribs) and an absolute minimum (no extra ribs). In any case there is an unmistakable tendency for the extra ribs to be either absent or completely developed.

Sex and rib expression

Previously we found that sex and occurrence of ribs were associated. It is now interesting to extend the inquiry to include the expression of the ribs as well. For this purpose asymmetry has been disregarded, and individuals are classified according to the total amount of rib present (table

TABLE 18

Distribution of sexes in grades of rib expression without regard to asymmetry.

COMBINATIONS R+L	GRADE RIB EXPRESSION	NUMBERS ♂♂	NUMBERS ♀♀	RATIO ♂/♀
00	0	369	195	1.89
01, 10	1	76	52	1.46
02, 11, 20	2	144	84	1.71
03, 12, 21, 30	3	161	131	1.23
04, 13, 22, 31, 40	4	163	168	0.97
14, 23, 32, 41	5	117	146	0.80
24, 33, 42	6	156	174	0.90
34, 43	7	110	169	0.65
44	8	195	416	0.47
Total		1491	1535	0.97

18). From the column of sex ratios, it is clear that there is a striking uniform trend from many males with low rib expression to few males with high rib expression. It appears that some factors, directly or indirectly associated with the female sex, cause the greater development of costal tissue at the thoraco-lumbar border in the mouse.

Sacral position and rib expression

The question is whether or not individuals, both male and female, that have an extra presacral vertebra also tend to have ribs more completely

developed. Tabulations made up by suitable groupings within table 17 are given for frequencies of overall grades of rib expression within the two principal categories of vertebrae in table 19. For testing purposes males and females are considered separately, and asymmetrical sacrums are grouped with the 27-psv class. The distributions as given are apparently

TABLE 19

Distribution of the population in grades of rib expression with regard to sex and number of presacral vertebrae. O-grade omitted. Analysis of variance for rib expression in vertebrae classes.

GRADE RIB EXPRESSION	psv, ♂♂		psv, ♀♀	
	26	27	26	27
1	69	7	46	6
2	137	7	70	14
3	145	16	108	23
4	147	16	129	39
5	90	27	94	52
6	122	34	109	65
7	77	33	89	80
8	103	92	160	256
Totals	890	232	850	535
Means	4.39	6.20	5.04	6.57

SOURCE OF VARIANCE	SUM OF SQUARES		DF	MEAN SQUARE
For males:				
Between vertebrae classes	601.92		1	601.92
Within vertebrae classes	5008.06		1120	4.47
Total	5609.98		1121	
	F=134.66	P<1%		
For females:				
Between vertebrae classes	761.64		1	761.64
Within vertebrae classes	5547.87		1338	4.15
Total	6309.52		1339	
	F=183.71	P<1%		

not normal in type. This fact may be related to biological processes, but is perhaps in part the result of the grading system, unit differences in grade not corresponding exactly with equal numbers of biological units from grade to grade.

In males the mean grades of rib development are 4.4 and 6.2 for the 26- and 27-psv types, respectively. In females the comparable means are 5.0 and 6.6. Both in males and in females the differences of the means are statistically significant as shown by the variance analysis of table 19. The

F-test for significance is taken from SNEDECOR (1937). Thus it seems that the grade of expression of the ribs within each sex is determined by factors which are associated with those which determine the presence of an extra presacral vertebra, though the female sex by itself or by factors associated with it is conducive to the more frequent production both of extra ribs and of an extra presacral vertebra.

SUMMARY OF ANALYSIS

If individuals are considered as the irreducible units, the factors causing the total variability in the position of the sacrum and the number of pairs of ribs may be apportioned as in columns A. If, however, the frequent occurrence of asymmetry of the sides of individuals is allowed for, an apportionment may be made as in columns B.

FACTORS COMMON TO:		PORTIONS OF TOTAL VARIABILITY			
		SACRUM		RIBS	
		A	B	A	B
Genetic	Sex	0.09	0.105	0.05	0.04
	Other	0.00	0.00	0.00	0.00
Non-genetic	Sibships	0.00	0.00	0.00	0.00
	Litters	0.08	0.095	0.17	0.14
	Individuals	0.83	—	0.78	—
	Both sides	—	0.62	—	0.54
	Single sides	—	0.18	—	0.28
Total		1.00	1.00	1.00	1.00

From these summaries it is clear that the most considerable portion of the total variation both of sacrum and of ribs is caused by the local action of non-genetic factors within individuals, and further, that about $\frac{1}{4}$ or $\frac{1}{3}$ of these factors act on single sides, independently of other agents. These factors are seemingly so intangible that they do not permit further resolution with the records available.

An examination of the records was made for the effect of tangible non-genetic factors operative upon the position of the sacrum. Age of mother, or some complex of factors associated with maternal age, is responsible for not more than 9/10 of the resemblance of litter mates. Other factors are unidentified.

For the most part the scale of factors which determine variability in the position of the sacrum has been treated as independent of the scale which determines the number of pairs of ribs. It is interesting, therefore, to determine whether or not the two regions are correlated. This may be done from a four-fold table made by dividing table 17 into 13- and 14-ribbed and

26- and 27-psv animals. This gives the frequencies of the four possible combinations of ribs and presacral vertebrae, 13/6, 14/5, 13/7, and 14/6, with the sexes separate in order not to confound the sex effect with the nongenetic factors (table 20). For both males and females the correlations between the thoraco-lumbar and the lumbo-sacral borders, even though subject to error because of paucity of 13/7 animals, indicate that about one-half of the factors act similarly on the two regions. More exactly, this may mean that the operations of some agents are sufficiently extensive along the vertebral column to include the two critical borders within their fields. This association for the mouse is the same as that found by KÜHNE (1936) for man, by TUFF and BERGE (1936) for the pig, and by GREEN (1939) for the rabbit.

TABLE 20

Correlation between variation at the thoraco-lumbar border and the variation at the lumbo-sacral border, sexes separate.

MALES	26-psv	27-psv	TOTAL	FEMALES	26-psv	27-psv	TOTAL
14-rib	890	232	1122	14-rib	805	535	1340
13-rib	361	8	369	13-rib	178	17	195
Total	1251	240	1491	Total	983	552	1535
	r=0.56				r=0.50		

Average, between borders, r=0.53

DISCUSSION

Throughout the analysis, the problem has been presented as dealing with structural and numerical variations in vertebrae. It should not be overlooked that these variations are but one manifestation of factors which probably operate early in prenatal development and include among their end-products variations of nerves, blood vessels, and muscles, as well as of bones. The evidence for this, while not abundant, is convincing. FREDE (1934) found that there was a very definite association between the nerves of the sacral plexus and the conformation of the sacrum in the rat, and further that the positions of the last intercostal nerves varied with the expression of the ribs. KÜHNE (1936) has reviewed the literature which establishes the interrelationships of vertebrae, muscles, and blood vessels. It is justifiable, therefore, to focus attention on the variations of ribs and sacrum, if we understand that the accessory influences which cause these variations actually operate upon fundamental embryonic processes.

What is the nature of the fundamental processes and what ancillary forces operate upon them?

For mammals the exact nature of the processes governing the development of complex organ patterns is highly speculative and can be surmised

only by analogy with the experimental analyses of development in other vertebrates and invertebrates, the interpretations of which are themselves controversial. The formation of somites and their subsequent differentiation into vertebrae and ribs presents a developmental pattern that may be controlled by inductive agents flowing into the axial region, by capacities for self-differentiation, or by various time-space combinations of induction and self-differentiation. Or the system may be thought of as controlled by growth and differentiation gradients. By assuming that these two gradients are somewhat distinct, various combinations of their individual velocities may account for the pattern differences in the end result (BACKMAN 1934). Thus if the growth gradient is higher through any part of the region of the vertebral column including the thoraco-lumbar and the lumbo-sacral borders, or if its highest point is earlier than that of the differentiation gradient, additional thoracic or lumbar vertebrae or both will be marked out of the primary mesenchyme. If the reverse conditions obtain, fewer vertebrae will result. The vertebrae, however, occur only in whole numbers, whereas the gradient combinations very likely form a continuous scale.

The precise nature of the developmental processes involved in vertebra formation is somewhat obscure, but we do know something of the genetic and non-genetic forces which operate upon these processes, at least in Bagg albino mice.

In the first place, it can be deduced from the experimental results that the field of action of the variable forces may extend over the thoraco-lumbar border only, or over the lumbo-sacral border only, but frequently the field extends over both borders (50 percent determination by common factors). It may be that the area of the field itself varies, that its position along the axis of polarity varies, or that there are serial combinations of size and position of the field.

In the second place, it is found that the sexes differ with respect to the potentiality for forming either 13 or 14 pairs of ribs and either 26 or 27 presacral vertebrae. Males are 13-ribbed about twice as frequently as females and have 27 presacral vertebrae about half as frequently. This same relationship occurs in several other species of mammals. There are at least two possible explanations of this bias. It may be the result of "skeletal" genes located upon the sex chromosomes but not concerned physiologically with the determination of sex. Females would have double sets and males single sets of such genes, on the assumption that the Y chromosome is practically inert. Or the sex factors themselves may impart different physiological characteristics to the developing embryo with respect to somite- and vertebra-forming potentialities. In any case, about five percent (ribs) to ten percent (presacral vertebrae) of all the factors acting upon the de-

velopment of the vertebral column are somehow associated with sex. Other genetic factors very likely have considerable effect in random bred stocks but in this stock their effect has been rendered constant by the long inbreeding.

In the third place, if we assume that the particular genetic composition of the Bagg albino stock of mice is such that each developing individual has originally the potentiality for forming either 13 or 14 pairs of ribs and either 26 or 27 presacral vertebrae and that the potentiality is partially restricted by the sex, then various combinations of tangible and intangible non-genetic, developmental factors must be responsible for the balance of the determination. Tangible non-genetic factors, those which act upon litters as units, have a small but measurable magnitude. In this case, age of the mother, or some complexity of non-genetic influences associated with age of mother, may be one of the essential variable factors. Size of the litter appears not to be. Intangible factors which act upon individuals as units are extremely important, comprising over 80 percent of all the variable factors. Of these about one-third (ribs) to one fourth (sacrum) are so localized in their action that they influence only single sides of individuals. Moreover, at the lumbo-sacral border in asymmetrical animals sacralization occurs on the right side on the 27th vertebra about half as frequently as on the left. This indicates that some factors act not randomly but preferentially upon single sides. No such bias in the asymmetry was found at the thoraco-lumbar border. This importance of intangible factors compares with that found in studies of variation in other isogenic stocks previously reported. WRIGHT (1934) found that an extra toe in the guinea pig is determined 41 percent by intangible factors; WRIGHT and CHASE (1936) for piebald spotting in the guinea pig, 89 percent; REED (1936) for harelip in the mouse, 73 percent. In swine, LUSH, HETZER, and CULBERTSON (1934) found that birth weights depended on intangible factors to the extent of 47 percent of the total variance.

The Bagg albino stock, itself practically isogenic, is very probably genetically different from other inbred stocks of mice, which differ from it in pairs of ribs and number of vertebrae. In view of the situation in this particular strain, it seems that extensive data from carefully controlled experimental breeding should be gathered before adducing a genetic factorial explanation for variations in the skeleton. The single factor explanation advanced by KÜHNE (1936) for similar variations in man cannot possibly by itself explain the variations in the mouse. Other authors (SHAW 1929, TUFF and BERGE 1936, FREEMAN 1939) have avoided simple genetic explanations for the comparable variations in swine, yet none have considered the possibility of non-genetic agents acting during development

which are evident in the experimental results for this particular strain of inbred mice.

SUMMARY

Variations in the vertebral column, involving extra ribs at the thoraco-lumbar border and an extra vertebra at the lumbo-sacral border, occur in so many species of mammals that it seems probable that no species of mammal is free of such variability.

Inbred stocks of mice differ from one another in their characteristic skeletal types. None of those examined was found to be wholly constant; several (P, C_3H, dba, C_{57}Blk, Leaden, N, A) produce non-modal types in small numbers; others (C) are about equally divided between two types; and others (Gates, L, Bagg albino) have several combinations of thoracic and lumbar vertebrae.

With respect to the factors causing variability in the position of the sacrum in the Bagg albino stock, sex factors, or factors associated with sex, account for 9 to 10.5 percent of the total; non-genetic factors common to litters, for 8 to 9.5 percent; other non-genetic factors specific within the individuals, for 80 to 83 percent.

Age of mother is responsible for a large part of the litter mate resemblance in sacral position.

Asymmetry of the sacrum occurs in 13.5 percent of the population, and the left innominate bone is anterior to the right about twice as frequently as the reverse.

With respect to factors causing variability in the occurrence of extra ribs, sex factors, factors operating on litter mates, and factors operating on individuals have about the same relative magnitude as similar factors causing variability in the position of the sacrum.

Higher grades of rib development occur in females; lower grades in males.

About 50 percent of the factors act over both the thoraco-lumbar and the lumbo-sacral borders. The remainder are presumably more restricted in field of action.

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OVAL BLOOD CELLS IN HUMAN SUBJECTS TESTED FOR LINKAGE WITH TASTE FOR PTC, MID-DIGITAL HAIR, HAIR COLOR, A-B AGGLUTINOGENS, AND SEX

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A FIELD study was undertaken in 1939 by WYANDT in order to collect human pedigrees showing oval red blood cells. The rare but non-pathological condition of elliptical erythrocytes was apparently known in medicine even before the first published account which we have been able to locate (DRESBACH 1904), but its hereditary nature was not well established until 1929 (HUNTER and ADAMS). CHENEY (1932) first reported the trait to be a simple Mendelian dominant on the basis of a three-generation pedigree, and STRAUSS and DALAND (1937) furnished confirmation from another three-generation pedigree. Several other cases of familial elliptocytosis have been reported subsequently. In a forthcoming paper WYANDT will publish pedigrees on oval cells in three family lines involving more than a hundred individuals. Her investigation confirms the dominant mode of unit inheritance suggested by previous less extensive studies.

During the course of WYANDT'S field study it was possible for her to gather data upon several additional traits that could be used as test factors in a search for linkage.

Taste for PTC (phenyl-thio-carbamide) was investigated by the use of filter paper dipped into a saturated acetone solution of the compound, dried, and cut into pieces 1 cm square, excepting in a family represented by two sibships to whom PTC crystals on the tip of a moistened tooth pick were presented.

The presence or absence of hairs over the middle phalanx of the fingers (exclusive of thumbs) was determined by examining both hands in daylight (with the exception of one sibship which was examined by electric light). An effort was made to detect fine, nearly invisible hair and to note the presence of hair follicles. When follicles were seen, hair was reported as present even if no hair was visible.

Hair color was matched at the back of the head, close to the scalp, to hair samples of standard shades (the "Shur-on" scale manufactured by Schwerner, Oppenheim Co., New York). Examination was made by daylight (except for one sibship which was seen by electric light).

Eye color was matched to the Martin-Schultz scale (J. F. Lehmanns Verlag, Munich). However, since only five families containing seven pairs of offspring were "crucial,"—namely, heterozygous families in which linkage could be expected to appear if it exists,—linkage tests for eye color could not be made with these data.

Classification into blood group A, B, or O was made from suspensions of cells in two percent sodium citrate in physiological saline, mixed with equal volumes of standard Group A and Group B sera in hanging drop preparations.

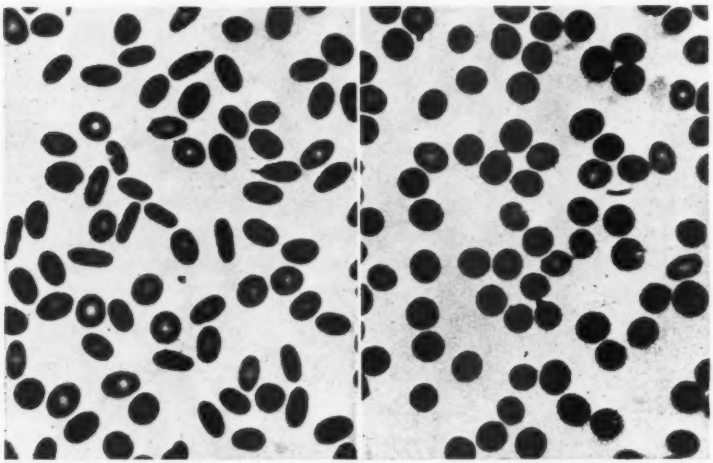

FIGURE 1.—Left, oval cells. Right, round cells.

For classifying individuals with respect to oval and round red blood cells, moist preparations were inspected under No. O cover slips, using small enough drops of blood so that a single layer of cells was obtained. One thousand cells were tabulated for each subject. The percentages of oval cells gave a dichotomous distribution, one group of subjects classified as "oval" showing percentages that varied from 72 to 96 and another group classified as "round" showing percentages that varied from zero to 10. Figure 1 shows micro-photographs of oval cells and round or normal cells.

The main method used for tests of linkage was that of "like" and "unlike" sibling pairs (BURKS and TOLMAN 1932, BURKS 1938, PENROSE 1935). FISHER's (1934–35) u statistic, although more sensitive, at least in the "single back-cross" mating, did not in general meet the requirements of the present study because of the irregularity of the mode of transmission of

the test factors (other than A-B-O groups and sex). The method which was used examines, in effect, the fourfold correlation between sibling-pair likeness-unlikeness on two traits and can be applied even if data from only a single generation are available. The method becomes more sensitive, however, if "non-crucial" sibships may be discarded, and consequently no sibships were retained that were clearly unsuited to test a linkage hypothesis (namely, families in which an oval-celled parent showed the recessive phenotype in the test factor), although sibships have been tabulated which may or may not be crucial (namely, a few sibships in which there is no offspring recessive for the test factor to prove the oval-celled parent doubly heterozygous).

In table 1 the data for the linkage tests consist of the "observed" entries, which show the number of sibling pairs who are "alike" on both traits, "unlike" on both, and "alike" on one but "unlike" on the other. Clearly in the absence of linkage, the ratio of the entries in the first two columns should equal the ratio of the entries in the third and fourth columns. The significance of deviations from proportionality was tested by chi-square (using FISHER's short-cut formula for the fourfold table and applying YATES's correction for continuity). The degree of deviation—in a direction favorable or unfavorable to a linkage hypothesis—was also calculated by the BOAS-YULEAN ϕ for a fourfold point distribution.

The data have also been examined as to evidence for "non-linkage," since failure to establish linkage in small population samples does not *ipso facto* disprove its existence. The tendency in human genetics has perhaps been too readily to label as negative data which do not stand up to tests of significance, without inquiring whether the data are adequate for establishing positive results if present. Though admittedly loose linkages would take more data to become established than we ordinarily obtain in field studies of human populations, it evidently has "mop-up" value to inquire whether pedigree findings are definitely inconsistent with close linkages. To this end we have set up "expected" distributions on certain assumptions with respect to recombination.

For simplicity's sake we have assumed zero crossing over in the case of mid-digital hair, for which both parents are often heterozygous, since under this condition (even if there is no recombination) sibling pairs will appear in all the four "expectation" categories of table 1 and thus make the chi-square test applicable. It is clear from the results of table 1 that even if recombinations significantly higher than zero were tested for,—for example, .10 or higher,—the results would still constitute strong evidence against linkage.

In the case of hair color a "non-linkage" test was not made, because the mode of inheritance of hair-color is not yet well enough agreed upon to

justify the setting up of an "expected" linkage distribution. With the remaining traits, for which only one parent is frequently heterozygous, we have set up expected distributions on the assumption of 25 percent re-

TABLE 1

Oval blood cells tested for linkage and non-linkage with five normal traits.

TEST FACTOR	BLOOD CELLS ALIKE		BLOOD CELLS UNLIKE		LINKAGE TEST			NON-LINKAGE TEST	
	ALIKE	UNLIKE	ALIKE	UNLIKE	DEV. OF OBSERVED DATA FROM CHANCE DISTRIBUTION			DEV. OF OBSERVED DATA FROM LINKAGE EXPECTATION	
					ϕ	χ^2 (n=1)	P	χ^2 (n=2)	P
Taste for PTC									
Observed	5	1	7	1	−.06	.30	.50–.70		
Expected (most fav. to linkage)	3.75	2.25	3.00	5.00				9.64	<.01
Expected (least fav. to linkage) (if c=.25)	5.53	.47	7.59	.41				1.57	.30–.50
Mid-digital hair									
Observed	10	10	18	10	−.14	.48	.50		
Expected (if c=0)	17.50	2.50	14	14				28.00	≪.01
Hair color									
Observed	10	8	13	11	.01	.05	.80–.90		
A-B-O groups									
Observed	16	13	17	24	.14	.79	>.30*		
Expected (if c=.25)	18.34	10.66	16.25	24.75				.88	.60–.70
Sex (oval fathers)									
Observed	9	10	18	12	−.12	.33	.50–.70		
Expected (partial sex if c=.25)	11.88	7.12	11.25	18.75				8.34	.01–.02
Sex (oval mothers)									
Observed	6	4	10	7	.01	.12	.70–.80		

* When the families contributing to the test of oval cells×A-B-O groups are separated into double and single-back-cross families and tested by FISHER's u_{11} and u_{31}, the fiducial chance of the results observed becomes approximately .06.

combination. As in the case of mid-digital hair, the chi-square test was used to test the observed data for their deviation from the hypothetical.

Though several of the fourfold distributions were based upon too few cases to permit a rigorous application of the chi-square test, the general conclusion reached by the chi-square test in such cases is probably sound: that the data neither establish nor disprove genetic linkage.

TASTE FOR PHENYL-THIO-CARBAMIDE

The data of BLAKESLEE and SALMON (BLAKESLEE 1932, BLAKESLEE and SALMON 1931) and of SNYDER (1931, 1932) give satisfactory evidence that inability to taste phenyl-thio-carbamide is transmitted as a simple recessive. The presence of modifiers, however, is suggested by the fact that "tasters" and "non-tasters" of the crystals or of filter paper treated with a saturated solution vary within their groups in thresholds of sensitivity to PTC solutions and show some overlapping between their groups (SETTERFIELD, SCHOTT and SNYDER 1936, HARTMANN 1939[1]). While occasionally the threshold of an individual changes significantly from one test to another (SALMON and BLAKESLEE 1935), PTC data offer results sufficiently clear-cut on the whole to permit tests for the detection of linkage.

Five sibships containing 13 offspring (none of whom happened to be young children) were used in the tests of linkage with oval cells. These yielded the 14 sibling pairs distributed in table 1 and showed no evidence of linkage.

The five sibships were then retabulated to show expectations when the distribution of oval- and round-cell offspring remains the same in each sibship but with a recombination ratio between the factors for oval cells and taste for PTC of .25. Since there were three families which could not be proved to have crucial genetic composition (even when data for parents were available), distributions were calculated upon hypotheses "most favorable" and "least favorable" to detection of linkage. For example, in one sibship the most favorable supposition was that the oval-cell parent was heterozygous for taste; the least favorable supposition, that he was homozygous dominant for taste.

When the "most favorable" supposition is used, the probability of a chance deviation from the linkage distribution of a magnitude as great as that obtained is less than .01. But when the "least favorable" supposition is used, the probability reaches the .30 to .50 range. In view of the small number of cases and the equivocal results, we should list this pair of traits as among those for which close linkage is neither established nor disproved.

[1] HARTMANN presents a bimodal curve for PTC threshold and shows that while all her series of non-tasters of crystals reach or exceed the threshold for solutions represented by the minimum frequency on the curve, so do one fifth of her series of tasters of crystals.

PRESENCE OR ABSENCE OF MID-DIGITAL HAIR

DANFORTH (1921) infers from his data that absence of hairs over the middle phalanx of the fingers may be due to one primary recessive. A forthcoming paper from the Genetics Record Office supports this view.

No restrictions as to age of subjects were made and none seem necessary, since the youngest subjects (age two or three) were found to have mid-digital hair present.

Ten usable sibships contained 33 offspring yielding 48 pairs as distributed in table 1.

No evidence in favor of linkage appears. The sibships were retabulated to show expectations when the distribution of oval- and round-cell offspring remains the same in each sibship, but with zero recombination between the factors for oval cells and mid-digital hair. When there was doubt as to the genetic composition of a family, the formula consistent with the distribution of offspring but least favorable to detection of linkage was assumed. For example, in one sibship of five offspring it was assumed that the mother, who was not seen, was heterozygous rather than recessive for mid-digital hair, since this would dilute the evidence obtainable from the doubly heterozygous father. Oval-cells and mid-digital hair were considered as alternating between coupling and repulsion in the doubly heterozygous parents of successive families, which presumably would yield an equitable result in the frequencies of "like" and "unlike" sibling pairs for a group of families even if not for individual families.

Since the expected frequency of the second entry is less than five, there is question as to the large contribution which this entry appears to make to chi-square. If we should arbitrarily reduce this contribution to the amount contributed by column 1, P would still be in the neighborhood of .01. Close linkage between oval cells and mid-digital hair, the more so because the "expected" values represent those least favorable to the detection of linkage, seems therefore to be ruled out; rarely would data in the presence of close linkage deviate so far from expectation by chance.

HAIR COLOR

Although the mode of transmission of hair color, and even the classification of hair color, are by no means generally agreed upon, the schema used by one of us in an earlier study of linkage in man (BURKS 1938) gave results sufficiently promising to justify its continued use for linkage tests until a better formulation is established through further research.

Only the dark-light series, presumably due mainly to melanic pigment, is here considered. Red shades are discarded, being too rare in this material to warrant separate treatment. Red tints are disregarded in brown hair,

the hair samples being judged only for darkness and lightness. Instead of classifying individuals upon an absolute scale, which would be an arbitrary procedure with a trait like hair color showing continuous variation in the general population, we (quoting from BURKS 1938) "followed the principle of classifying sibling pairs as 'similar' in hair color if their shades were so close together on the chart that discrimination between the two shades of hair was doubtful or even liable to a reversal of direction if repeated on another day. All other sibling pairs were counted 'dissimilar'." The previous result of using such a classification was consistent with the hypothesis that the dark-light series of hair color is mainly determined in a single gene locus corresponding to a number of non-dominant alleles, giving "(1) in families with two heterozygous parents, four potential phenotypes corresponding to four genotypes [H_1H_3, H_1H_4, H_2H_3, H_2H_4] and (2) in families with one homozygous parent (or families in which certain phenotypes are not readily distinguished), two potential phenotypes. . . ."

No offspring younger than fifteen years were used in the tabulations, since up to about this age rapid changes in hair color are taking place. Individuals with gray hair were of course not included. Eight sibships containing twenty-seven usable offspring yielded the forty-two pairs distributed in table 1.

Like the previous tables of observed data, this one gives no evidence in favor of linkage. For reasons already explained, hair color was not tested for "non-linkage."

A-B AGGLUTINOGENS

The generally accepted theory of the main blood groups as due to three allelemorphs, A and B dominant with respect to their absence, but not with respect to each other, has been employed in the interpretation of data. Siblings were classified as "alike" or "unlike" in the blood agglutinogens, but only with respect to the alleles possessed by their doubly heterozygous parent. For example, in one sibship the oval-celled parent was B, the round-celled parent A, so A was merely treated as "not-B" and would have been grouped with O if any O's had appeared. Since more families were available here than were available for the other test factors, only families known to be "crucial" (by the criteria of parental combinations and traits appearing in offspring) were included in the tabulations.

Using twelve sibships, containing 37 offspring yielding 44 pairs, gave the data shown in table 1.

This is the first indication of a positive test; although P is not very low, we should bear in mind that the probability of getting a chance deviation in a direction favorable to linkage is approximately half this magnitude.

A procedure similar to that used with the other traits was employed to

build up a hypothetical linkage table for a recombination ratio of .25. Most of the usable families were "double back-cross"—that is, had one doubly heterozygous parent and one doubly homozygous parent—which greatly simplified the calculations. In the three families having both parents heterozygous for blood type A, the first was assumed to be "most favorable" (in coupling and repulsion conditions) for the detection of linkage, the second, "least favorable," and the third, "most favorable."

The calculations indicate that in spite of the possible exaggeration of the requirements for close linkage, the probability that distributions consistent with close linkage would deviate through chance by an amount as great as that observed is .60 to .70. Thus the two complementary sets of calculations are both consistent with close linkage, although they by no means establish it.

The results looked interesting enough (and the segregation of oval cells and of A-B agglutinogens regular enough) to warrant a reworking of the data by FISHER's (1934–35) $\underline{u}$ test—namely, for the double back-cross families having an expected 1:1:1:1 ratio of offspring in the absence of linkage:

$$u_{11} = (a-b-c+d)^2 - (a+b+c+d)$$

and for the single back-cross families having an expected 3:3:1:1 ratio of offspring:

$$u_{31} = (a-b-3c+3d)^2 - (a+b+9c+9d)$$

where a, b, c, and d are frequencies of offspring of four types within a family. For twelve double back-cross families the sum of the $\underline{u}_{11}$ was 18 with S.E. 15.9; for three single back-cross families the sum of the $\underline{u}_{31}$ was 22 with S.E. 20.8. Combining the two sets of data gives a $\underline{u}$-score of 40 with S.E. 26.2, the ratio of $\underline{u}$ to S.E. being 1.53. If the sampling distribution of $\underline{u}$ is something like normal, the probability of a chance result as favorable to linkage would be about .06.

To rule out the possibility that correlation between oval cells and the blood groups in the general population might simulate a "linkage distribution," a contingency table was set up for oval and round blood cells against blood groups A, B, and O (there were too few AB's to include). A chance distribution was very closely approximated, chi-square corresponding to P in the range .50 to .70.

INCOMPLETE SEX LINKAGE

Since the appearance of HALDANE's (1936) article on incomplete sex linkage in man, it has been clear that all genes which appear at first sight to be autosomal must be tested for possible locus in the homologous segment of the X Y chromosome pair. The unique attribute of an incompletely

sex-linked character is that it transmits from the father preferentially to offspring of the same sex as the father's affected parent. When, as in the present data, it is frequently not known which of the paternal grandparents was affected, an indirect test (less sensitive than a direct test) can be made by ascertaining whether individual families show a tendency for affected offspring to be of the same sex.

Only the families in which the father transmits the trait are crucial for detecting incomplete linkage, but we have worked up the data on offspring of oval-celled mothers as well as fathers so that one tabulation could serve as a control upon the other.

With oval-celled father nine sibships containing 32 offspring yielding 49 pairs were available.

With oval-celled mother there were eight sibships containing 24 offspring yielding 27 pairs.

No evidence for linkage appears. The supplementary calculation (for the cases having oval-celled fathers) based on a hypothetical distribution for recombination .25, moreover, leaves us with reasonable assurance that oval-cell is not incompletely sex-linked with a crossover ratio less than .25.

DISCUSSION AND SUMMARY

A systematic collection of data on "test factors" was made during the course of a field study of families characterized by the appearance of oval blood cells. It is obviously a desirable procedure in any study conducted to establish or corroborate the mode of transmission of a human trait to include at the same time a search for linked traits. It is to be hoped that such a search will be carried out routinely in future studies, not only with the test factors here employed, but with a larger number of test factors as these become available.

Tests previously reported for the detection of linkage between random pairs of traits have generally given results consistent with chance recombination—both because recombination is in fact chance (two given traits having only one chance in twenty-four of belonging to the same linkage group) and because the number of pedigrees has often been too small to give clear-cut detection of linkage even if present. The investigations on record have commonly failed to distinguish between the reasons for negative results, with the consequence that many of the data laid aside with "no evidence for linkage" might well be reexamined as to "evidence for non-linkage." In the present study evidence for non-linkage as well as for linkage was considered by testing the observed data for their deviation from hypothetical linkage tables as well as from chance distributions. The hypothetical linkage tables were set up for a recombination ratio of zero excepting when this gave expectations close to zero in certain categories, in

which case a recombination ratio of .25 was arbitrarily assumed. The method could obviously be extended to cover any desired recombination ratio.

Certain refinements over the method here used would also be useful, although we believe the broad conclusions drawn out of the data are valid. In particular, it would be desirable: (1) to devise a way of dealing systematically with the sibships which may or may not be "crucial" for detecting linkage (as when one parent may be either simplex or duplex for the test factor); (2) to devise a method for allowing for irregularities in the transmission of traits mainly but not wholly attributable to a single gene substitution; (3) to gain more light upon the appropriateness of the chi-square test when sibships supply more than one pair of siblings. It seems clear that in the presence of linkage, sibling pairs in a sibship larger than two cannot be considered as independent. On the other hand it is not immediately clear (though MATHER 1938, p. 118, recommends it) that all pair-combinations provided by sibships larger than two can be treated as independent in the absence of linkage.

If, as we believe, these reservations do not seriously influence the results of the present study, it seems justifiable to conclude that close linkage may be ruled out for oval cells with mid-digital hair and with sex (that is, evidence is against incomplete sex linkage), that the data are equivocal for oval cells with taste for phenyl-thio-carbamide and with hair color (probably negative for the latter), and that the possibility of linkage between oval cells and the A-B agglutinogens deserves further investigation.

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THE STABILITY OF BROKEN ENDS OF CHROMOSOMES IN ZEA MAYS

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I. INTRODUCTION

IF CHROMOSOMES are broken by various means, the broken ends appear to be adhesive and tend to fuse with one another 2-by-2. This has been abundantly illustrated in the studies of chromosomal aberrations induced by X-ray treatment. It also occurs after mechanical rupture of ring-shaped chromosomes during somatic mitoses in maize and is assumed to occur during the normal process of crossing-over. In a previous publication (McCLINTOCK 1938b) it was shown that following breakage of a single chromatid in a meiotic anaphase of maize, fusion occurs at the position of breakage between the two sister halves of this broken chromatid. Because of this fusion, the two sister halves cannot separate freely from one another in the following mitotic anaphase. As the two centromeres of the terminally united chromsomes pass to opposite poles in this mitotic anaphase, a chromatin bridge is produced. The tension on the bridge configuration, following the poleward migration of the centromeres, results in rupture. Once again, a chromatid with a broken end enters each sister telophase nucleus. The questions then arise: Will fusions occur at the position of breakage between the two sister halves of each of these broken chromosomes giving rise to an anaphase bridge configuration in the following mitosis? If so, will this breakage-fusion-bridge cycle continue in each successive nuclear division, or will the broken end, produced by the rupture of an anaphase bridge configuration, eventually "heal," thus discontinuing the breakage-fusion-bridge cycle? Answers to these questions were presented briefly in a preliminary publication (McCLINTOCK 1939). The results presented in this latter publication and those presented in this paper have led to the following conclusions. (1) If a chromosome, broken at the previous meiotic anaphase, is delivered to the primary endosperm nucleus through either the male or the female gametophyte, the breakage-fusion-bridge cycle will continue in the successive nuclear divisions during the development of the endosperm tissues. (2) A similarly broken chromosome delivered to the zygote nucleus by either the sperm or the egg does not give rise to bridge configurations in successive nuclear divisions in the sporophytic tissues. The broken end heals. There is a complete cessation of the breakage-fusion-bridge cycle. (3) The breakage-fusion-bridge cycle is confined to the gametophytic and endosperm tissues of the generation immediately following the initial break in the chromosome. (4) Healing of the

broken end in the embryonic sporophyte is permanent. When a chromosome with a healed broken end is introduced into gametophytic or endosperm tissues in succeeding generations, no fusions of broken ends result either between sister halves of the broken chromosome or between two such broken chromosomes when both are introduced into a single nucleus. It is the purpose of this paper to present the evidence for these conclusions.

II. THE TYPES OF GENETIC VARIEGATION PRODUCED BY THE BREAKAGE-FUSION-BRIDGE CYCLE

If a broken chromosome continued the breakage-fusion-bridge cycle in successive nuclear divisions, its presence should be made evident by genetic variegation in endosperm and plant tissues. This would follow when the

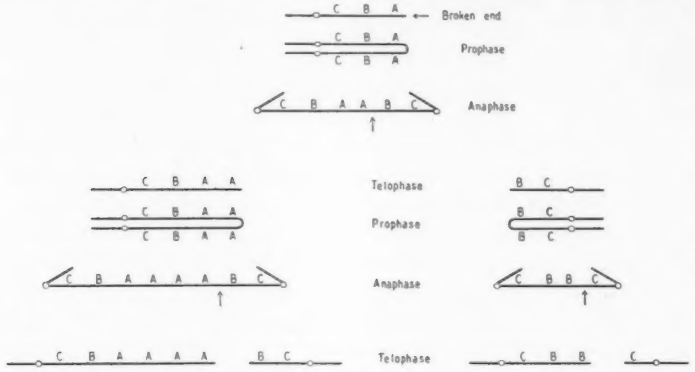

FIGURE 1.—A representative illustration of the method by which variegation may be produced in tissues carrying a chromosome with a broken end. The clear circle represents the centromere. The dominant genes *A B* and *C* are carried by the arm with the broken end, *A* being near the broken end and *C* near the centromere. The homologue of this chromosome (not diagrammed) is considered to be normal and to carry the genes *a b* and *c*. Division of this broken chromosome results in fusion at the position of breakage between the two split halves (prophase, second diagram from top). This is followed by a bridge configuration in the following anaphase (anaphase, third diagram from top). The arrow points to the position of breakage, the two broken chromosomes entering the sister telophase nuclei (telophase, right and left). This process is repeated in successive divisions. One such division is diagrammed below each of these two telophase chromatids. The diagrams illustrate how dominant genes may be deleted or reduplicated followed the breakage-fusion-bridge cycle.

broken chromosome carried dominant genes and its normal homologue carried the recessive alleles. Figure 1 illustrates the method by which variegation is produced in consequence of the breakage-fusion-bridge cycle. The line at the top of the figure represents a chromosome with a broken end. The dominant genes *A B* and *C* are carried by the arm with a broken end, *A* being close to the broken end and *C* nearest to the centromere. The

diagram immediately below represents this chromosome at the following prophase, fusion having occurred between the two sister chromatids at the position of previous breakage. Separation of the two sister centromeres at anaphase results in a bridge configuration (third diagram, fig. 1). If a break occurred at the position of the arrow, a chromosome carrying the genes *A A B C* and possessing a broken end would enter one telophase nucleus (left in the diagram) and one chromosome carrying the genes *B* and *C* would enter the sister telophase nucleus (right in diagram). All the cells arising from this latter cell would lack the dominant gene *A*. Thus, the recessive gene *a* would appear in all cells arising from this cell, and the process which results in variegation would have commenced. Each of these broken chromosomes could, in turn, repeat the process just outlined. In the telophase chromosome to the right, the broken ends of the two sister chromatids would again be fused at the succeeding prophase (see diagram), and a bridge configuration would result at the following anaphase (see diagram below). If a break occurred at the arrow, a broken chromosome carrying *B B C* would enter one telophase nucleus and a broken chromosome carrying only *C* would enter the sister nucleus. All the cells arising from this latter cell would have lost the dominant genes *A* and *B*, and the tissues would show the character of the recessive alleles *a* and *b*. In the first telophase to the left, a similar process has been diagrammed whereby the dominant gene *A* is lost to one daughter nucleus and repeated duplications of *A* genes are introduced into the sister nucleus.

The diagram (fig. 1) is merely an example. The break in the first anaphase might have occurred between the two *A* genes. Several nuclear cycles might take place before a break occurred to one side of these genes resulting in the loss of the *A* gene to one of the daughter nuclei. On the other hand, the first or successive breaks might have occurred close to one of the centromeres resulting in the loss of all three dominant genes to one nucleus and their duplication in the sister nucleus. Variegation patterns of dominant and recessive tissue resulting from this type of behavior should be very distinctive. Some tissues could be totally recessive—that is, *a b c*. In this case, all three genes would be lost from a nucleus in a single anaphase break. Other tissues could be recessive for *a* but variegated for *b* and *c*. In this case *A* would be lost from the cell following one anaphase break, while *B* and *C* would be subsequently lost. In these tissues, patches should be found which are (1) *a b c* where *B* and *C* have subsequently and simultaneously been lost; (2) *a b C* from which *B* and not *C* has been lost. In this latter patch, which is wholly *a b* in genetic character, still smaller patches should be found which are *a b c*. No tissues should be found which show the genetic constitution *a B c*. In other words, when variegation within variegation is present, the developmental pattern should show the

loss of the terminal genes before loss of genes close to the centromere. Since variegation of endosperm tissues was the means by which individuals possessing broken chromosomes were detected, a description of the types of variegation observed will be given after the method has been described by which broken chromosomes may be obtained.

In order to secure cytological evidence of the presence of a broken chromosome in a plant and to study the genetic consequences of its behavior, two methods were employed. Both methods involve the breakage of chromosome 9 at a meiotic anaphase and its deliverance to the endosperm and the zygote after successful passage through the developmental periods of the male or the female gametophytes (the pollen grain and embryo sac, respectively). This necessitates the production of a broken chromosome

FIGURE 2.—Photomicrograph of chromosome 9 at pachytene in a microsporocyte. The centromere appears as a grey bulge (arrow). The short arm of one of the homologues terminates in a small knob. Note the deep-staining region adjacent to the centromere in the short arm and the smaller, more widely spaced chromomeres in the distal part of the arm. The proximal deep-staining segment appears relatively longer in this photograph than in the diagrams given in this paper. This is due to an overlapping of the two homologous chromosomes immediately distal to the junction of the deep-staining and lighter-staining regions.

which possesses at least a full set of genes if transmissions are to occur through the male gametophyte, or broken chromatids with only relatively short terminal deficiencies if transmissions are to occur through the female gametophyte. No deficiencies within the short arm of chromosome 9 which are transmitted through the pollen are known, but deficiencies of terminal segments up to and including one third of the short arm may be transmitted through the female gamete (McCLINTOCK unpublished).

Both methods involve the use of abnormalities in the structural arrangement of chromosome 9 of maize. A photograph of a normal chromosome 9 at the mid-prophase of meiosis is given in figure 2. The genes *Yg*[2], *C*, *Sh*, and *Wx* are located in the short arm of chromosome 9. *Yg* normal green plant, *yg* yellow-green plant; *C* colored aleurone, *c* colorless aleurone; *Sh* normal development of the endosperm, *sh* shrunken endosperm; *Wx* normal starch staining blue with iodine, *wx* waxy starch staining red with iodine). The linear order of these genes is *Yg-C-Sh-Wx*. *Yg* is located very

near the end of the short arm. *C* is located approximately a quarter of the distance in from the end of the short arm. *Sh* is located very close to it. *Wx* is located at approximately the middle of the short arm, although its exact position has not been determined. It should be noted that the gene *Yg* is a plant character, whereas the genes *C*, *Sh*, and *Wx* are endosperm characters.

III. THE PRODUCTION OF A BROKEN CHROMOSOME BY MEANS OF A REARRANGEMENT IN CHROMOSOME 9

(a) *Description of the rearrangement*

The first method will be described in considerable detail, since it affords particularly favorable material for a cytological and genetical study of the behavior of broken chromosomes. It involves the use of a chromosome 9

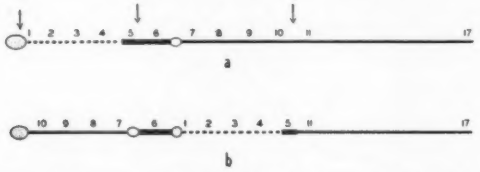

FIGURE 3.—A normal chromosome 9 terminating in a large knob (stippled). The clear oval region represents the centromere. The individual parts of the chromosome have been numbered from 1 to 17. The broken line (segments 1 to 4) represents the widely spaced small chromomeres of the distal two-thirds of the short arm. The wide line (segments 5 and 6) represents the proximal deep-staining region of the short arm adjacent to the centromere. The long arm is represented by a narrow line. The arrows point to the positions of breaks which resulted in the rearranged chromosome shown in b.

with a moderately complex rearrangement of parts which arose following X-ray treatment. A normal chromosome 9 is diagrammed in a, figure 3. In the strain of maize irradiated, the short arm terminated in a large knob (stippled). (In certain strains of maize no knob is present at the end of the short arm. In other strains small or intermediate size knobs are found. The knob substance lengthens the chromosome but does not carry essential genic material.) The proximal one-third of the short arm (fig. 2 and regions 5 and 6 fig. 3) adjacent to the centromere (clear oval region in diagram) is characterized by large, closely associated deeply-staining chromomeres, suggesting heterochromatin in appearance. It will be referred to as the pycnotic region. The distal two-thirds of the short arm (dash lines fig. 3) is composed of small, widely spaced chromomeres. The arrows in a (fig 3) indicate the positions of breaks which resulted in the rearrangement of segments shown in b (fig. 3). The large terminal knob has been broken into two unequal parts, the small part together with three quarters of the short arm being inserted into the long arm in the normal order. The section which

includes 6 to 10 became attached to the larger segment of the knob at region 10, resulting in an inverted order for the genes in this segment.

(b) *Types of chromosomes produced as the result of crossing-over*

Plants which contain one rearranged chromosome 9 and a normal homologue give rise to several types of anaphase I and II configurations following crossing over in the rearranged segments. A diagram of the meiotic prophase synaptic configuration is given in figure 4. The regions where crossing over results in aberrant configurations are labelled A, B, and C. Region A includes the segment from 1 to 5. Region B includes segment 6. Region C includes the segment 7 to 10.

Several types of altered chromosomes result from crossing over in these regions. In figure 5, the types of dicentric chromatids produced by crossing

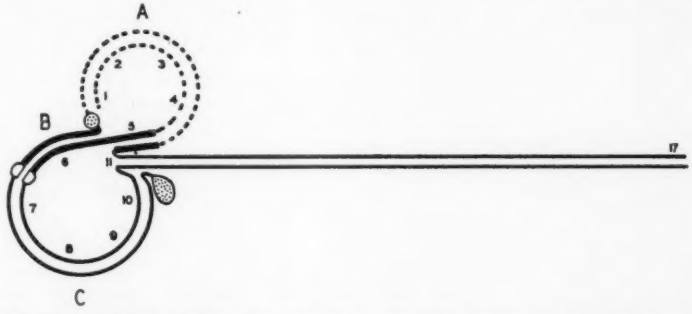

FIGURE 4.—Synaptic configuration produced following homologous associations of a normal chromosome 9 (without a knob) and the rearranged chromosome diagrammed in b of figure 3. Region A includes segments 1 to 5. Region B includes segment 6 (between the small knob and the centromere). Region C includes segments 7 to 10.

over are diagrammed. The type of crossing over which produces each type of dicentric chromatid is given in the description beneath the figure. In most cases, the dicentric chromatid produces a bridge configuration in anaphase I. The two types of crossovers which lead to bridge configurations at anaphase II are indicated in the legend. In addition to the dicentric chromatids, two new monocentric chromatids can be produced as the result of crossing over. These are diagrammed in figure 6. The various types of acentric chromatids, which are the complements of the dicentric chromatids, are mainly lost to the successive nuclei following crossing over. Thus, they have not been diagrammed.

As indicated in figure 5, most of the dicentric chromatids result in a bridge configuration in anaphase I. Only the three-strand double crossovers involving regions A and B result in bridge configurations in anaphase II.

Observations of the number of bridge configurations in the two meiotic divisions indicate the frequency of the types of crossovers. Among 238

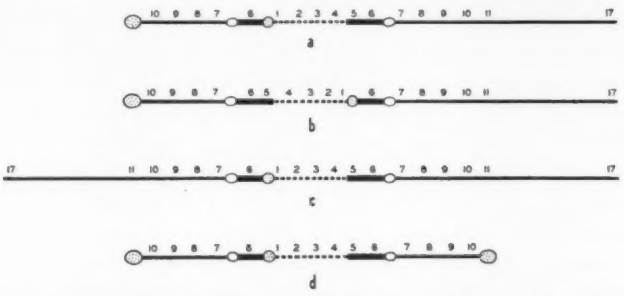

FIGURE 5.—Types of dicentric chromatids produced as the consequence of crossovers in regions A, B, and C of figure 4. a. Dicentric chromatid produced following a single crossover in region A. A bridge configuration occurs at anaphase I. b. A dicentric chromatid resulting from a two-strand double crossover involving regions A and B or A and C. A bridge configuration occurs at anaphase I. c. Dicentric chromatid resulting from a three-strand double crossover involving one chromatid of the rearranged chromosome and the two chromatids of the normal chromosome. One crossover in region A, the other in region C, will result in a bridge configuration in anaphase I. If the second crossover is in region B, a bridge configuration will occur in anaphase II. d. Dicentric chromatid resulting from a three-strand double crossover, as in c, but the chromatids involved are the reverse—that is, one from the normal chromosome and two from the rearranged chromosome.

microsporocytes in anaphase I, 77 or 32 percent showed a chromatid bridge. In two of these microsporocytes a double bridge with two acentric fragments was observed—the result of a four-strand double crossover.

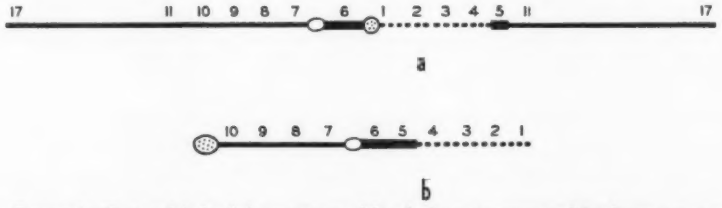

FIGURE 6.—Types of derived chromosomes with a single centromere resulting from a crossover in regions B or C of figure 4. The chromatid in a could be recovered, since it contains a complete set of genes plus a duplication of segments 11 to 17. The chromatid in b could not be recovered, since it is deficient for the segments 11 to 17.

In anaphase II, only those dyads were counted in which both sister cells could be observed at mid-anaphase. Among 271 such dyads, only five or 1.8 percent showed a chromatin bridge configuration in one of the sister cells.

The dicentric chromatid most frequently formed (a, fig. 5) is of particular significance for this study. The part of the dicentric chromatid extend-

ing from the small internal knob to the right end of the chromosome is the equivalent of a complete chromosome 9. Left of the small internal knob is the short pycnotic region 6 followed by the second centromere and the segment 7 to 10 which terminates in the large knob. At anaphase I, the two centromeres moving in opposite directions cause the chromatid to break. Analyses of anaphase I configurations and of the composition of this chromosome in microspores indicate that the break may occur at any position between the two centromeres. Breaks which occur immediately to the right of the small internal knob or to the left of this position, will result in one broken chromatid with at least a full set of genes of chromosome 9. This chromatid could result in a viable spore since no essential genic material has been lost from the chromosome at this initial break. Breaks which occur to the right of the small inner knob within regions 1 to 6 would result in a chromatid deficient for various numbers of genes of the short arm, depending in each case upon where the break had occurred. Regardless of where the break occurs, the resulting broken chromatid to the left would be deficient for a large number of genes of chromosome 9. It would not be expected to produce a functional spore. Interest centers, therefore, on the transmissions of the broken chromatid to the right.

The two dicentric chromatids shown in b and c (fig. 5) could give rise to broken chromatids with a full complement of genes. Those in d would not be expected to survive, because both of the broken chromatids would be highly deficient. In b (fig. 5) the surviving chromatid following breakage would have most of the genes in the short arm in the inverted order. In c (fig. 5) the surviving broken chromatid could have its genes in the normal order if the break occurred between the knob and the left centromere and would be indistinguishable from the broken chromatid derived from a. If the break occurred in segment 6 adjacent to the right centromere, the surviving broken chromatid would have the order of the genes reversed, as in b. Since none of the transmitted broken chromosomes so far obtained have shown an inverted order of genes, attention will be concentrated on the surviving broken chromatid arising from the dicentric chromatid of a.

(c) *Types of kernels resulting from the cross*

$$\frac{C\ Sh\ Wx \text{ rearranged chromosome } 9}{c\ sh\ wx \text{ normal chromosome } 9} \times c\ sh\ wx \text{ normal chromosome } 9.$$

The dicentric chromatid (a, fig. 5) arises from a crossover in region A of figure 4. This region carries the loci of the genes *Yg C Sh* and *Wx*. Since the chromosomes with a broken end arise from a dicentric chromatid which suffered a break at anaphase I or II, all surviving chromosomes with a broken end should be crossovers within this region A. If the breakage-fusion-bridge cycle continues after the initial meiotic anaphase break, the

endosperm tissue should be variegated when it has received from one parent a broken chromosome 9 carrying any or all of the dominant endosperm genes and from the other parent a normal chromosome 9 with the recessive alleles, *c sh* and *wx*.

The following tests were made in order to show that the variegated kernels arise following a crossover in region A of figure 4. Plants were obtained which possessed a rearranged chromosome 9 carrying the genes *Yg C Sh Wx* and a normal chromosome 9 with the genes *yg c sh wx*. These plants were crossed to those carrying two normal chromosomes 9 each with the genes *yg c sh wx*. The types of single crossovers within this region and the resulting dicentric chromatids with their genic constitutions are shown in figure 7. The rearranged chromosome 9 with the genes *Yg C Sh Wx* and the normal chromosome 9 with the genes *yg c sh wx* are diagrammed at the

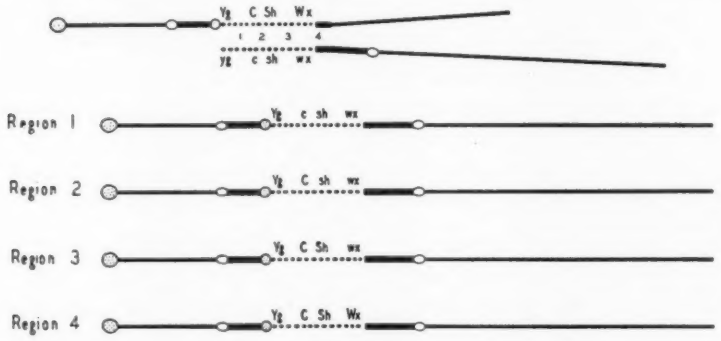

FIGURE 7.—The genic constitution of dicentric chromatids resulting from crossing over in segments 1 to 5. At the top of the diagram, homologous associations of the rearranged chromosome 9 and a normal chromosome 9 are represented only between segments 1 to 5. The genic constitution of each chromosome is indicated. Between the two chromosomes the regions where crossovers may be detected are numbered 1, 2, 3, and 4. The dicentric chromatids with their genic constitutions resulting from crossovers in each of these regions are given below.

top of figure 7. To simplify visualization of the crossovers, homologous associations of these two chromosomes are diagrammed only in the region carrying these genes (region A of fig. 4). There are four marked regions of crossing over, designated 1, 2, 3, and 4 in the diagram. The amount of crossing over which occurs between two normal chromosomes 9 in regions 1, 2, and 3 are 19 percent, 3 percent, and 21 percent, respectively (EMERSON, FRASER, and BEADLE 1935). The normal amount of crossing over in region 4 (from *Wx* to the break) cannot be stated, since there were no previous tests for this region. A crossover in region 1 (from *Yg* to *C*) would give rise to a dicentric chromatid carrying the genes *Yg c sh wx*. Following

the meiotic anaphase break, a surviving broken chromatid with the endosperm genes *c sh* and *wx* could be delivered to the primary endosperm nucleus. Since no variegation would result, this broken chromatid could not be detected by the endosperm characters.

A crossover in region 2 would give rise to a dicentric chromatid carrying *Yg C sh wx*. If the first break in the dicentric chromatid occurred to the left of *C*, a broken chromosome carrying the genes *C sh* and *wx* could be delivered to an endosperm, provided the successive breaks in the gametophyte divisions did not eliminate *C*. The resulting endosperm should be homozygous for *sh* and *wx* but variegated for *C* and *c*.

A crossover in region 3 would give rise to a decentric chromatid carrying *Yg C Sh wx*. Thus a broken chromosome with the genes *C Sh* and *wx* could be delivered to the primary endosperm nucleus. The endosperm tissue which develops should be homozygous for *wx* but variegated for *C-c* and *Sh-sh*.

A crossover in region 4 would give rise to a dicentric chromatid carrying the genes *Yg C Sh* and *Wx*. If the original break and the successive breaks in the gametophyte divisions occurred to the left of *C*, a broken chromosome with the genes *C Sh* and *Wx* would be delivered to the endosperm. The endosperm then should be variegated for all three genes, the detectible order of the loss of the dominant genes being *C*, followed by *Sh*, followed by *Wx*. In this last case, a break occurring between *C* and *Sh*, either in the first or in subsequent gametophyte divisions, could deliver a broken chromosome with the genes *Sh* and *Wx* to the endosperm tissues. The endosperm would then be *c* but variegated for *Sh-sh* and *Wx-wx*. If the original or subsequent break in the gametophyte occurred between *Sh* and *Wx*, the endosperm tissues would be *c* and *sh* but variegated for *Wx-wx*. Thus, single crossovers in regions 2, 3, and 4 should be genetically detectible by the type of endosperm variegation. Since *C* and *Sh* are close together and the genes *Sh* and *Wx* relatively distant, there should be many more *C-c Sh-sh wx* kernels than *C-c sh wx* kernels. If the normal relative rates of crossing over for these two regions is maintained in the plants heterozygous for the altered chromosome 9, the two types of variegated kernels should occur in the ratio of 1 to 7. That this ratio is maintained is seen from an examination of table 1, which gives the types of kernels resulting from the cross *C Sh Wx* rearranged chromosome 9/*c sh wx* normal chromosome 9 × *c sh wx* normal chromosome 9. This table considers only the endosperm characters. In the crosses contributing to table 1, the plant character *yg* need not be considered. However, in some crosses, both parents were homozygous *Yg*. In other crosses, the heterozygous parent was either *Yg Yg*, or *Yg yg* as in figure 7, while the recessive parent was homozygous for *yg*.

In this table it may be seen that 99 percent of the kernels have the genet-

ic constitution of the two parental chromosomes of the heterozygous individual—that is *C Sh Wx* non-variegated or *c sh wx*. Only 0.8 percent of the kernels in the first column and 0.51 percent of the kernels in the second column are variegated, but the relative proportions, respectively, of the variegated kernels representing crossovers in regions 2, 3, and 4 are similar

TABLE 1

Types of kernels arising from the cross.

$$\frac{\textit{C Sh Wx}\text{ rearranged chromosome 9}}{\textit{c sh wx}\text{ normal chromosome 9}} \times \textit{c sh wx}\text{ normal chromosome 9}$$

	♀ PARENT HETEROZYGOUS		♂ PARENT HETEROZYGOUS	
Non-variegated, non-crossover kernels				
C Sh Wx	11,716		6,789	
c sh wx	11,679		5,812	
Subtotal		23,395		12,601
		98.9%		99.1%
Non-variegated, crossover kernels				
C sh wx	2		3	
c Sh Wx	3		1	
C Sh wx	12		21	
c sh Wx	8		14	
C sh Wx	0		0	
c Sh wx	1		0	
Subtotal		26		39
		0.19%		0.30%
Variegated kernels				
C-c sh wx	7		7	
C-c Sh-sh wx	78		48	
C-c Sh-sh Wx-wx	21		11	
c Sh-sh Wx-wx	2		0	
c sh Wx-wx	1		0	
Subtotal		109		66
		0.80%		0.51%
Totals	23,530		12,706	

in both crosses. Among the variegated kernels 14 were homozygous for *sh* and *wx* but variegated for *C-c*. These represent crossovers in region 2. One hundred twenty-six were homozygous for *wx* but variegated for both *C-c* and *Sh-sh*. These represent crossovers in region 3. Thirty-two were variegated for all three genes. These represent crossovers in region 4. The relative proportions of crossovers in regions 2 and 3 conform to expectancy on the basis of both the cytological and genetic evidence for the distance between the genes *C Sh* and *Wx*. The relative proportion of crossing over

from *Wx* to the break in the rearranged chromosome 9 could not be anticipated, since no previous genetic evidence was available for this region.

In both crosses there were a few kernels (0.19 percent in column 1 and 0.30 percent in column 2) carrying crossover chromatids which did not show variegation. According to expectancy, these should represent double crossover chromatids with normal, non-broken ends. The kernels should possess either a normal chromosome 9 or a rearranged chromosome 9 but not a broken chromosome 9. Whether, in any class, a normal or a rearranged chromosome is present would depend upon the positions of the two crossovers with respect to both the genes and the chromatids involved (see table 6).

Cytological evidence for the conclusion that the variegated kernels carry a chromosome 9 with a broken end and that the non-variegated kernels possess a non-broken chromosome could be obtained readily by examination of the chromosome complement of the plants arising from each of these types of kernels. However, before these results are described, it is desirable to emphasize the genetic evidence which allows one to conclude that the breakage-fusion-bridge cycle is responsible for the variegation in the endosperm. These may be enumerated as follows. (1) The frequency of the different types of kernel variegation indicates their relationship to crossing over in region A, figure 4, which, in turn, results in a dicentric chromatid and finally a broken chromosome. (2) The very low percentage of non-variegated kernels carrying crossover chromatids. This is expected on the assumption of their origin from a double crossover. These should be relatively infrequent. (3) The developmental order of loss of the genes in the variegated kernels containing *C Sh* and *Wx* is the type expected on the basis of the breakage-fusion-bridge cycle as outlined in figure 1. The genes nearer the broken end are lost before those nearest the centromere. (4) A striking confirmation of the breakage-fusion-bridge cycle appears in the various colored regions in the variegated kernels. In normal endosperm tissues the *C* allele may be present in a single dose (*C c c*), a double dose (*C C c*), or a triple dose (*C C C*). Usually the depth of color of the aleurone layer is related to the number of *C* alleles present, *C c c* being lighter than *C C c* which in turn is lighter than *C C C* (JONES 1937). When a broken chromosome 9 is introduced in the cross outlined, the depth of color in the *C* regions in the variegated kernels varies from very light to exceptionally dark. The regions are distributed in well defined patches for each intensity of color. This type of variegation is exactly what should be produced following the breakage-fusion-bridge cycle. As illustrated in figure 1, genes may not only be lost but may be reduplicated as a consequence of this cycle. Thus, various doses of the *C* allele in different regions of the aleurone are to be expected.

On the basis of the genetic evidence obtained from the variegated kernels, it was expected that the plant tissues would likewise show variegation for the *Yg* gene when the homozygous parent contained the gene *yg*, as well as the genes *c sh wx*, in its two normal chromosomes 9 while the heterozygous parent contained *Yg* in each of its two chromosomes 9. Some of the crosses contributing to table 1 were of this type. The plants arising from the variegated kernels would be expected to show variegation (*Yg-yg*) or to be wholly *yg* if the *Yg* locus had been lost either in the original or in an early subsequent break. The results were unexpected. The plants were either *Yg* or *yg*. There were no variegated individuals. Forty-eight plants were grown from the variegated kernels. Forty were *Yg* and eight were *yg*. Cytological examination showed that all the plants arising from these variegated kernels actually possessed a broken chromosome 9. The *Yg* plants had at least a complete chromosome 9. All the *yg* plants had lost a segment at the end of the short arm of chromosome 9 which included the *Yg* locus. In all cases, however, the broken end of the chromosome 9 had healed in the somatic diploid tissues, discontinuing the breakage-fusion-bridge cycle and thus the possibility of producing variegation in the *Yg* individuals. If the non-variegated crossover kernels possessed a non-broken end resulting from a double crossover, none of the plants arising from these kernels should be *yg*. All should possess either the normal chromosome 9 or the rearranged chromosome 9. Twenty-six plants were grown from the crossover non-variegated kernels. All twenty-six were *Yg;* none was *yg*. Cytological examination showed that all possessed an unbroken chromosome 9.

The cross outlined above allows one to distinguish between the crossover types which give a broken chromosome 9 and those which give a non-broken chromosome 9. As stated earlier, the single crossovers in region 1 of figure 7 cannot be detected by variegation, because the broken chromosome has the constitution *c sh wx*. Since this region between the knob and *C* in normal material shows as much crossing over as that between *C* and *Wx*, a number of broken chromosomes must have remained undetected in the above crosses. In order to detect the transmissible broken chromosomes following a crossover in region 1, as well as in the other three regions, individuals of the constitution *C Sh Wx* rearranged chromosome 9/*C sh wx* normal chromosome 9 were crossed with plants carrying two normal chromosomes 9 each with *c sh wx*. In this cross, the single crossovers in region 1 (fig. 7) would result in a dicentric chromatid with the constitution *C sh wx*. Following breakage of the dicentric chromatid, the surviving broken chromosome should have the constitution *C sh wx* and would result in a kernel variegated for *C-c* but homozygous for *sh* and *wx*. The single crossovers in regions 2, 3, and 4 would produce the same types of variegated kernels as

described in figure 7. Since single crossovers in regions 1 and 2 result in surviving broken chromosomes with similar constitutions, the class *C-c sh wx* among the variegated kernels should be relatively increased in this cross. This is shown in table 2, where the results of this cross are recorded. The first column gives the results obtained when the female parent was heterozygous. The second column gives the results obtained when the male

TABLE 2

Types of kernels arising from the cross.

$$\frac{C\ Sh\ Wx \text{ rearranged chromosome } 9}{C\ sh\ wx \text{ normal chromosome } 9} \times c\ sh\ wx \text{ normal chromosome } 9$$

	♀ PARENT HETEROZYGOUS	♂ PARENT HETEROZYGOUS
Non-variegated kernels		
C	4,532	4,116
c	1	8
Variegated kernels		
C-c sh wx	16	38
C-c Sh-sh wx	17	22
C-c Sh-sh Wx-wx	4	6
Totals	4,570	4,190
Percentage variegated kernels	0.8	1.6

parent was heterozygous. This cross was designed to test the maximum number of surviving broken chromosomes. Because some of the double crossover chromatids in the *C* non-variegated class could not be detected, classification of kernel types in the non-variegated classes have not been included in the table. Kernels with the constitution *c* should arise only following mutation of *C* to *c*, which is very rare, or following deletion of the *C* gene from the broken chromosome before its deliverance to the endosperm as a consequence of the breakage-fusion-bridge cycle. The one *c sh wx* kernel in the first column was diseased. It is possible that color did not develop because of this disease. In the second column, six of the eight *c sh wx* kernels appeared on two of the 11 ears examined. This strongly suggests that they arose following contamination. Plants have not been grown from these kernels to check the chromosome constitution.

The results of a similar cross which included *yg* are given in table 3. This cross was designed to test for the presence of *yg* in the plants derived from the various types of kernels. If the variegated kernels are the only ones which have a broken chromosome, no *yg* should appear in plants arising from the non-variegated kernels, either in the non-crossover classes or the crossover classes. However, *yg* could appear in the plants derived from the

variegated kernels. No *yg* plants appeared in more than 400 plants derived from each of the two non-variegated, non-crossover classes. The three plants derived from the non-variegated crossover kernels were likewise *Yg*. Their chromosome 9 constitution showed that a double crossover chromatid with a normal end had entered the endosperm and zygote nuclei. Nine

TABLE 3

Types of kernels arising from the cross.

$$\frac{Yg\ C\ Sh\ Wx \text{ rearranged chromosome 9}}{Yg\ C\ sh\ wx \text{ normal chromosome 9}}\ ♀ \times yg\ c\ sh\ wx \text{ normal chromosome 9} ♂$$

Non-variegated, non-crossover kernels	Number of kernels
C Sh Wx	2,061
C sh wx	2,016
Non-variegated, crossover kernels	
C Sh wx	1
C sh Wx	1
Variegated kernels	
C-c sh wx	3
C-c Sh-sh wx	8
C-c Sh-sh Wx-wx	1

plants were obtained from the variegated kernels. Five of these were *Yg* and four were *yg*. All nine plants carried a broken chromosome 9. The five *Yg* plants possessed at least a complete chromosome 9. Each of the four *yg* plants possessed a chromosome 9 deficient for a terminal segment of the short arm which included the *Yg* locus.

(d) *The chromosome 9 constitution of plants arising from the variegated kernels*

In the previous section, genetic evidence has been given which indicates that variegation in the endosperm tissue is due to the presence in these tissues of a chromosome 9 with a broken end. The variegation appears as the consequence of the breakage-fusion-bridge cycle in successive nuclear divisions. In contrast, the plants arising from the variegated kernels, although possessing a chromosome with a broken end, do not show variegation. The broken end of the chromosome heals in the embryo nuclei and remains permanently healed regardless of the tissue in which it may later be present.

In this section it is desired to describe the types of broken chromosomes which are present in the plants arising from the variegated kernels. In a previous publication (McCLINTOCK 1938b) it was shown that a chromo-

some broken at a meiotic anaphase gave rise to a bridge configuration in the following microgametophyte division as the result of the fusion at the position of breakage of the two sister halves of the broken chromatid. If this occurred in both the male and the female gametophytes, the broken chromosomes delivered to the zygotes should include various types of deficiencies and duplications (see fig. 1), depending upon where the breaks occurred in the anaphase bridge configurations in the successive gametophyte divisions. If the broken end, produced as the result of a bridge configuration in the last gametophyte division, healed in the zygote nucleus, all the cells of the plant would show the same type of modified chromosome 9. The meiotic prophases in these plants would then reveal the constitution of the broken chromosome 9 which each plant had received.

One hundred twenty-six plants arising from variegated kernels were examined at meiotic prophase for the constitution of the chromosome 9 delivered by the heterozygous parent. In 120 of these plants, this chromosome 9 terminated in a broken end. In three plants the chromosome 9 delivered by the heterozygous parent had undergone a secondary modification; two involved a translocation between chromosome 9 and a second chromosome, and in the third plant the chromosome 9 was in the shape of a ring. In the three remaining plants, heterofertilization had occurred. In each of these three cases, sperms from two pollen grains had contributed to the development of the kernel, a sperm from one pollen grain fusing with the polar nuclei and a sperm from a second pollen grain fusing with the egg nucleus. A normal, unbroken chromosome 9 carrying genes not corresponding with those in the endosperm tissues was present in these plants. In maize, hetero-fertilization may be expected in a low percentage of the cases (SPRAGUE 1932). For comparison, 49 plants arising from the *C Sh Wx* nonvariegated kernels of table 1 were examined. All 49 plants had received the rearranged chromosome 9 with an unbroken end (b, fig. 3) from the heterozygous parent.

In 109 of the 120 plants possessing a chromosome 9 with a broken end no duplications were present of the type which could arise as a consequence of the breakage-fusion-bridge cycle in the preceding gametophytic divisions. As illustrated in figure 1, a chromosome with a broken end but possessing a duplication or repeat duplications of terminal segments could be present in these plants if the breaks in anaphase in the sucessive gametophytic divisions (two in the male gametophyte, three in the female gametophyte) did not occur at position of previous fusions—that is were non-median. To recover such a duplication, it is likewise necessary that following such a nonmedian break the longer segment be included in the telophase nucleus which will give rise to a gamete (first telophase to left in fig. 1 instead of first telophase to right). For descriptive purposes, therefore, the type of

recovered broken chromosome may be referred directly to the dicentric chromatid (a, fig. 5) from which it originally arose. This chromatid is reproduced in figure 8. In all cases except one (dash-line arrow, fig. 8) the recovered broken chromosome had the constitution *to the right* of the position

FIGURE 8.—Types of broken chromosomes which were recovered in plants arising from variegated kernels. In each plant, the type of recovered chromosome may be referred to the dicentric chromatid illustrated here. The arrows and brackets give the positions of the broken ends. The recovered broken chromosome had the constitution to the right of the arrow (except for the dash-line arrow) or to the right of any particular position within the bracket. In the case of the dash-line arrow, the recovered broken chromosome had the constitution to the left of the arrow. The numbers associated with the arrows or brackets indicate the number of plants which possessed this particular broken chromosome constitution.

of breakage as indicated by the arrows or to the right of any particular position within the brackets. The numbers placed above or below the arrows and below the brackets give the number of individuals with the constitution indicated. In the one exceptional case, the recovered broken chromosome had the constitution to the left of the dash-arrow in figure 8. This case is of particular interest and will be considered separately elsewhere in this paper.

Thirty-two of these plants showed a broken chromosome 9 with a section of the pycnotic region 6 extending beyond the small knob. In 40 plants, the broken chromosome ended in a small knob, the last break having occurred immediately to the left of the knob in most cases but through the knob in a few cases. In 17 plants, the chromosome was complete to the knob, but the knob itself was missing. In 19 plants, the recovered broken chromosome was deficient. The deficiencies ranged from loss of a single chromomere to loss of the entire short arm. In the latter case, the broken end terminated in the centromere.

Among the remaining 11 plants of the 120, the broken chromosome 9 possessed various types of duplicated segments arising in each case through secondary fusions and breakages. The broken chromosome 9 in no two plants was exactly alike, but for descriptive purposes the 11 plants may be divided into five classes according to the composition of the broken chromosome. In four of the five classes, the recovered broken chromosome showed evidence of only a single fusion and breakage following the original break at a meiotic anaphase. In the fifth case, at least two fusions and breaks followed the original meiotic break (type V, fig. 9). As will be shown later there appears to be a tendency for successive breaks to occur at positions of previous fusions, indicating a weak fusion of sister chroma-

tids following a break. A strong union is produced, however, following many of the breaks. Therefore, although the breakage-fusion-bridge cycle may have continued in all gametophyte divisions following the initial break at meiotic anaphase, the recovery of many complicated duplications

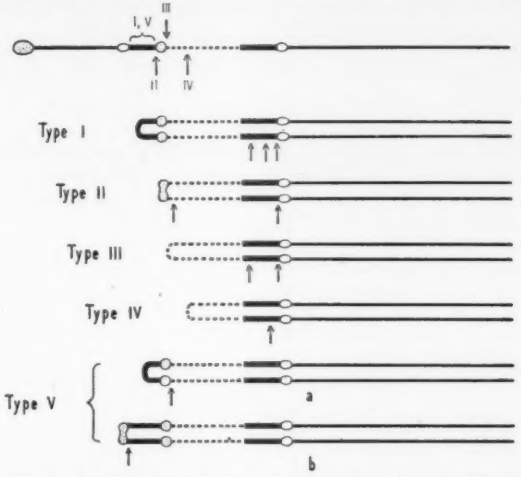

FIGURE 9.—The types of recovered broken chromosomes in plants arising from variegated kernels which demonstrate the breakage-fusion-bridge cycle. The dicentric chromatid from which each recovered broken chromosome originated is given above. Although the position of the initial break in this dicentric chromatid is not definitely known for each recovered broken chromosome, for purposes of illustrating the chromatin constitution of the broken chromosome, the break which preceded the final break in types I to IV and in a of type V may be referred to the dicentric chromatid (section of dicentric chromatid to right of arrows and bracket). This broken chromosome, with its two halves fused at the position of previous breakage, is reproduced below for each type. For types I to IV, the position of the final break is indicated by the arrows, one for each plant within a type, the broken chromatid with the upper centromere being recovered in the zygote. In type V, the recovered broken chromatid indicated that three successive breaks had occurred. The position of the first break is indicated in the dicentric chromatid. The position of the second break is indicated by the arrow in a, the chromatid with the upper centromere being in the line of descent. The position of the third break is indicated by the arrow in b, the recovered chromosome having the constitution of the broken chromatid with the upper centromere.

resulting from this process need not be anticipated. For illustrative purposes, the origin of the various classes of broken chromosomes with duplicated segments may be referred to the original dicentric chromatid with only one fusion and consequent break following the original meiotic break in types I to IV, and two successive fusions and breaks in type V. In figure 9, the dicentric meiotic chromatid in which the first break occurred is diagrammed above, the region of the first break being indicated by the arrows and the bracket for each of the five types. In all five types, the chromatid in the line of descent is that to the right of the break. This chromatid with its

two split halves fused at the position of breakage is diagrammed below for each type. The position of the second break is indicated by arrows, one for each plant in a type class except for types I and II, where the second break occurred close to the centromere in two cases in each of these two classes. The chromatid with the upper centromere was recovered in each case and represents the observed broken chromosome. Four plants were included in type I, three in type II, and two in type III. There was only one plant in

TABLE 4

The broken chromosome 9 constitution of plants derived from variegated kernels. The symbol (♀) or (♂) placed beside the kernel type indicates which parent was heterozygous for the rearranged chromosome 9.

KERNEL CHARACTER	CHROMOSOME ENDS IN KNOB	PYCNOTIC EXTENSION BEYOND KNOB	DUPLICATION OF SHORT ARM	NO KNOB, NO DEFICIENCY	TERMINAL DEFICIENCY	OTHERS
C-c Sh-sh Wx-wx (♀)	7	6	0	1	0	1
C-c Sh-sh Wx-wx (♂)	3	2	0	1	0	1
C-c Sh-sh wx (♀)	15	17	5	7	12	1
C-c Sh-sh wx (♂)	11	5	4	6	5	1
C-c sh wx (♀)	2	1	0	1	0	0
C-c sh wx (♂)	2	2	1	1	0	0
c Sh-sh Wx-wx (♀)	0	0	0	0	2	0
Totals	40	33	10	17	19	4*

* For description of these types, see text.

type IV. The recovered broken chromosome in this plant possessed a deficiency of approximately one-fifth of the distal segment of the short arm but possessed a duplication of nearly all of the remaining proximal four-fifths of the short arm. As stated above, in type V the breakage-fusion-bridge process is detected through one more cycle. The upper chromatid was in the line of descent following each of the two successive breaks.

Although the breaks in the dicentric chromatids may occur at any position between the two centromeres the evidence from all 120 cases suggests that there is a tendency for the breaks to occur at either side of the small inner knob. In approximately half of the cases, the composition of the recovered broken chromosome indicated that a break had occurred to either side of this knob (fig. 8). Similarly, in six of the 11 plants with duplicated segments (fig. 9) one or more of the breaks must have occurred to one side of the knob. In the chromosome represented in type V (fig. 9) both the second and the third breaks must have occurred adjacent to the knob. The results of these studies on the chromosome 9 constitution of plants arising from variegated kernels are given in table 4 and are summarized in table 5.

The tendency for successive breaks to occur at the positions of previous fusions probably accounts for the relatively few cases of complicated duplications which otherwise would be expected to be present following repeated fusions and breaks in the gametophyte divisions. Likewise, this tendency should work toward increasing the correspondence in the genic constitution of the broken chromosome in the endosperm and the plant tissues. The two sperms (one for the endosperm tissues and one for the plant tissues) need

TABLE 5

A summary of the various types of broken chromosomes 9 delivered to the zygote through the female and male gametophytes.

TYPE OF BROKEN CHROMOSOME	THROUGH FEMALE GAMETOPHYTE		THROUGH MALE GAMETOPHYTE	
	NUMBER OF PLANTS	%	NUMBER OF PLANTS	%
Chromosome ends in knob	24	30.7	16	35.5
Pycnotic extension beyond knob	24	30.7	9	20.0
Duplication of short arm	5	6.4	5	11.1
No knob, no deficiency	9	11.5	8	17.7
Terminal deficiency	14	17.8	5	11.1
Others	2	2.5	2	4.4
Totals	78		45	

not possess the same genic constitution with respect to the genes *C Sh* and *Wx*. A decidedly non-median break in a bridge configuration at the division of the generative nucleus could introduce a duplication of one or more genes into one sperm nucleus and a deficiency of these genes in the sister sperm nucleus. Following such a break, the genic constitution of the endosperm and embryo tissues would not be the same. Similarly, this reasoning may be applied to the embryo and endosperm tissues when the broken chromosome is introduced through the female gametophyte.

Among the examined plants the genic constitution of the broken chromosome 9 in the plant tissues was determined in all cases and compared with the genic constitution of the broken chromosome 9 delivered to the endosperm of the kernel from which the plant arose. Excluding the three cases of hetero-fertilization, as previously mentioned, correspondence was obtained in all but two cases. In these two cases, the male was the heterozygous parent; the endosperm and embryo nuclei differed in genic constitution. In both cases, the chromosome 9 delivered to the endosperm possessed the genes *C* and *Sh* as shown by *C-c* and *Sh-sh* variegation. The chromosome 9 in the plant tissues lacked both these genes because the

broken chromosome 9 was deficient for all of the short arm, in one case, and nearly all of it in the second case.

It will be noted in the summary table (table 5) that the proportion of the various types of recovered broken chromosomes are similar whether delivered through the sperm or the egg nucleus. It is known that deficiencies of the extent observed in these studies are not transmitted through the pollen when the tube nucleus possesses such a deficient chromosome. A deficient broken chromosome was observed in five plants when the broken chromosome was delivered through the male parent. This deficiency must have arisen through a non-median break in an anaphase bridge configuration subsequent to the division which produced the tube nucleus.

A deficient broken chromosome was observed in 14 plants when the broken chromosome was delivered through the female gametophyte. When all the nuclei of a female gametophyte possess a short terminal deficiency of chromosome 9, a functional embryo sac can develop. It is not certain, therefore, whether the deficiency in the broken chromosome arose at meiosis or in a subsequent division. In two cases, the evidence suggests that the deficiency may have arisen at the meiotic anaphase, for both the endosperm and the plant nuclei were deficient for terminal segments of the short arm of chromosome 9 which had deleted the same genes.

The long duplications observed in plants which have received their broken chromosomes from the male parent have some theoretical interest. All such duplications must arise in divisions subsequent to that which gave rise to the initial break—that is, subsequent to the meiotic break. This subsequent break must have occurred at a non-median position in an anaphase bridge. If the long duplications originated in the first microspore division, the generative nucleus would receive the duplication and the tube nucleus would receive a highly deficient chromosome. Such a pollen grain would not be expected to function unless the tube nucleus had received an acentric fragment with a genic complement covering the deficiency. If the long duplications originate in the division of the generative nucleus, the two sperm nuclei would differ in constitution with regard to the genes *C Sh* and *Wx*. In the four cases of recovered long duplications, the detectible genic constitution of the embryo and the endosperm were similar. This would suggest that the duplication observed in these plants could have arisen during the first division of the zygote as a consequence of an unequal break in a bridge configuration. Since it is known that acentric fragments occasionally are carried through several nuclear cycles before being lost to the telephase nuclei, it is not possible to determine directly by this analysis whether a bridge configuration actually occurred at the first zygotic anaphase. If a bridge configuration does occur at this division, healing of the broken end must occur very shortly thereafter.

Mention should be made of the four plants arising from variegated kernels whose chromosome 9 constitution was unexpected (see table 4). One of these cases is of particular interest. As stated above, all but one of the recovered broken chromosomes arising from the original dicentric chromatid, possessed the broken segment to the right of the arrow or bracket in figure 9. However, one broken chromosome possessed the segment of the dicentric chromatid to the left of the dash-line arrow in figure 8. This broken chromosome, introduced by the male parent, is deficient for a large section of chromosome 9. It is known that a pollen grain, all of whose nuclei carry such a deficient chromosome, does not develop normally and does not produce a functional grain. It is highly probable that the acentric fragment, produced during the formation of the dicentric chromatid, was included in the microspore nucleus and again included in the tube nucleus following the first microspore division (McCLINTOCK 1938b). This acentric fragment could possess the genic material which is absent in the broken chromosome. A pollen grain with such a tube nucleus, possessing at least a complete complement of genes of chromosome 9, could develop normally and function in the delivery of two deficient sperms to the endosperm and zygote nuclei, respectively.

The genes *C Sh* and *Wx* were carried by this broken chromosome 9 (fig. 10). The normal chromosome 9 in this plant carried the recessive alleles, *c sh* and *wx*. (The observed order of loss of the dominant genes in the vari-

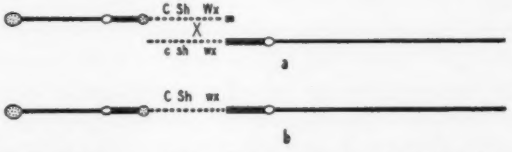

FIGURE 10.—a. Homologous associations of segments 1 to 4 of a broken chromosome (left of dash-line arrow, figure 8) and a normal chromosome. The genic constitution of each chromosome in indicated. A crossover, as indicated, would give rise to the dicentric chromatid shown in b.

egated endosperm of the kernel from which this plant arose was *Wx*, followed by *Sh*, followed by *C*. This is expected on the assumption of the breakage-fusion-bridge cycle, since the gene nearest the broken end (*Wx*) would be lost before the gene nearest the centromere (*C*).) Functional gametes carrying the dominant genes can be produced by this plant only following crossing over. All single crossovers in regions indicated by homologous associations in a (fig. 10) will give rise to dicentric chromatids (b, fig. 10) similar in constitution to that of a in figure 5. This dicentric chromatid is broken at anaphase I. Consequently, following such a crossover, all chromosomes with dominant genes must possess a broken end. In the cross of this plant with one homozygous for *c sh* and *wx*, these broken chromosomes

should give rise to kernels with variegated endosperms. Unfortunately, at the time this plant was ready for pollination, no plants homozygous for *c sh* and *wx* were available. Consequently, it was selfed. Pollen from this plant was also placed on silks of plants of the constitution *C c*. Among the 54 kernels obtained from the self, 12 showed the presence of the dominant gene *C*. The endosperms of all 12 kernels were variegated for *C* and *c*. (2 *C-c sh wx*:8 *C-c Sh-sh wx*:2 *C-c Sh-sh Wx-wx*). The remaining 44 kernels were completely recessive for all three endosperm genes.

The cross onto the heterozygous plant (*C c*) gave 1070 *C* non-variegated to 859 *c* to 133 *C-c* variegated kernels. The contribution from the female parent would lead to a 1:1 ratio of *C* non-variegated to *c*. Any distortion of this ratio would depend upon the *C* contribution of the male parent. Since variegation for *C-c* produced by the presence of a broken chromosome 9 carrying *C* and introduced by the male parent, can express itself only following union with *c* carrying nuclei contributed by the female parent, this class of 133 kernels may be added to the 859 *c* kernels to obtain the minimum number of *c* carrying gametophytes produced by the female parent. The resulting ratio of 1070 to 992 is close to the 1 *C*:1 *c* ratio which the female parent alone should contribute. The distortion in favor of the *C*-non-variegated class could be accounted for by the functioning of *C*-carrying gametes whose chromosome 9 possessed a normal end following a double crossover. In the material available, this class could not be detected. Nevertheless, the results of these two crosses are of significance in confirming the relationship between broken chromosomes and variegated endosperms.

The second plant of the four arising from the variegated kernels whose chromosome 9 constitution was unpredicted possessed a segment of chromosome 9 in the shape of a ring. This ring carried the genes *C* and *Sh* but not *Wx*. The variegation for *C-c* and *Sh-sh* in the kernel from which this plant arose was probably related to the aberrant mitotic behavior of the ring-shaped chromosome (McCLINTOCK 1938a). The origin of this ring chromosome was not determined.

The third plant possessed a translocation between the short arm of chromosome 9 and chromosome 8. The meiotic prophase figures were too poor to allow an analysis of this translocation.

The fourth plant had a complete chromosome 9 with the addition of a segment of chromatin of undetermined origin extending beyond the end of the short arm. The extra segment did not represent a duplication of part of the short arm of chromosome 9.

With the exception of the three cases of heterofertilization and the three cases just mentioned, all the other plants examined (120) showed the presence of a broken chromosome 9 in the plants derived from kernels with variegated endosperms. In 38 cases, the composition of the broken chromo-

some clearly indicated that it had arisen from the dicentric chromatid diagrammed in a, of figure 5 (arrows to the left of the small knob, fig. 8; dashline arrow, fig. 8; types I and V, fig. 9). All other cases could readily be derived from this dicentric chromatid as illustrated in figures 8 and 9.

That the variegation is the result of the breakage-fusion-bridge cycle is strongly supported by the types of recovered chromosomes illustrated in figure 9. It is not understood why this process is limited to the gametophyte and endosperm and does not continue in the embyro tissues. In all cases, the broken end of chromosome 9 healed in the embryo tissues. This is a permanent healing, for when this chromosome is again introduced into endosperm tissues in successive generations, it causes no variegation. When two such chromosomes are brought together in a single plant after each has passed through a sporophytic generation, no fusions occur between the broken ends. If the broken chromosome has a full set of genes or in addition a pycnotic extension composed of region 6, its behavior and transmissions through successive generations are comparable in every way to a normal, unbroken chromosome 9 (see f of this section).

(e) *The chromosome 9 constitution of plants arising from the non-variegated crossover kernels*

Earlier in this paper the assumption was made that the non-variegated kernels in a crossover class (tables 1 and 3) had received a non-broken chromosome 9 from the heterozygous parent as the consequence of a double crossover. Because no broken end was present, no variegation should be expected. In the classes *C sh Wx*, *c Sh Wx*, *C Sh wx*, and *c sh Wx*, the broken chromosome 9 derived from a double crossover chromatid could be either the normal chromosome 9 or the rearranged chromosome 9, depending up on where the two crossovers had occurred. The chromosome 9 constitution of 50 plants arising from the non-variegated crossover kernels are summarized in table 6. In 44 individuals, the chromosome 9 constitution was as expected—that is, the plant possessed either a normal chromosome 9 or a rearranged chromosome 9. Among the six individuals with exceptional constitutions, four arose following heterofertilization and consequently must be eliminated from consideration. In one of the two remaining exceptional individuals, a segment of the end of the long arm of chromosome 4 had been translocated to the end of the short arm of chromosome 9. The origin of this modified chromosome is not understood. The other exceptional individual showed a chromosome 9 with the same constitution as the dicentric chromatid in a, of figure 5, except that the left centromere was deleted. It is possible that this centromere was torn from the dicentric chromatid during anaphase I, the broken ends fusing on each side of the tear to give rise to the observed chromosome.

Genetic tests were conducted with some of these plants to verify the cytological determination of the presence of a double crossover chromosome. Plants of the constitution *Yg C Sh Wx* rearranged chromosome 9/*yg c sh wx* normal chromosome 9 were crossed by plants of the constitution *Yg c sh wx* normal chromosome 9. Since the locus of *yg* is almost immediately adjacent to the terminal knob in the normal chromosome 9

TABLE 6

Type of chromosome 9, delivered by the heterozygous parent, in plants arising from the non-variegated crossover kernels. The symbol (♀) or (♂) placed beside the kernel type indicates the heterozygous parent.

KERNEL CHARACTER	NORMAL CHROMOSOME 9	REARRANGED CHROMOSOME 9	OTHERS
C Sh wx (♀)	5	6	2†
C Sh wx (♂)	8	4	2*
c sh Wx (♀)	6	2	0
c sh Wx (♂)	3	5	1*
C sh wx (♀)	1	0	0
C sh wx (♂)	1	0	1*
c Sh Wx (♀)	0	2	0
c Sh Wx (♂)	1	0	0
Totals	25	19	6

* Endosperm and embryo chromosome constitution not alike due to hetero-fertilization.
† See text for description of chromosome 9 in these two plants.

(CREIGHTON 1934, MCCLINTOCK unpublished), crossovers between *yg* and the knob are infrequent. If the kernels in the non-variegated crossover classes possessed a chromosome derived from a double crossover chromatid, all the plants arising from these kernels which have a normal chromosome 9 should be heterozygous for yellow-green (*Yg yg*). Those possessing a rearranged chromosome 9 should be homozygous dominant (*Yg Yg*). These tests were conducted with nine plants carrying a normal chromosome 9 and four plants carrying a rearranged chromosome 9. The results conformed with expectancy.

(f) *The genetical and cytological behavior of the broken chromosomes in successive generations. Further proof that variegation follows the introduction of a recently broken chromosome into the endosperm tissues*

To test whether the broken chromosomes, summarized in table 5, would induce variegation in the endosperm tissues of the next succeeding generation, plants in all the categories of table 5 were either selfed or crossed by those containing two normal chromosomes 9 carrying *c*. In all of these tests

the broken chromosome carried *C*, its normal homologue, *c*. In the first category of plants in table 5,—that is plants whose broken chromosome ended in a knob,—the selfing of 28 plants gave rise to 6,142 *C* non-variegated kernels, 2,048 *c* kernels, and 3 that were variegated for *C* and *c*. Two plants were crossed by plants homozygous for *c*. There resulted 242 *C* non-variegated kernels, 249 *c* kernels, and no variegated kernels. The variegation in the three kernels appearing in the selfed progenies need not be related to the behavior of the original broken end of chromosome 9. A small percentage of such kernels have been noted in many genetic crosses in maize produced through causes the exact nature of which cannot be easily ascertained. Thus, the behavior in successive generations of this broken chromosome in column 1 of table 5 is comparable in every way to a normal chromosome 9 with a normal end.

Eighteen plants in the second category of table 5 were selfed. These plants possessed a duplication of the pycnotic region 6 of chromosome 9 extending beyond the small knob. The selfing of these eighteen plants gave rise to 4,209 *C* non-variegated kernels, 1,566 *c* kernels, and no variegated kernels. In no individual case was there a marked deviation from a 3:1 ratio, although in the total counts there is a slight deviation in favor of the *c* class. This is probably due to a slight selection against the pollen grains carrying the chromosome 9 with the pycnotic extension. The backcross ratios likewise suggest this. Pollen carrying *c* was placed on the silks of seven of these plants. There resulted 1,532 *C* non-variegated kernels, 1,565 *c* kernels, and one *C-c* variegated kernel. When pollen of two of these plants was placed on the silks of seven plants homozygous for *c*, there resulted 1,087 *C* non-variegated kernels, 1,200 *c* kernels, and no variegated kernels. A lack of variegated kernels in the progeny of this second category of plants likewise shows the stability of the broken end following its healing in the embryo of the parent plant. Similar tests were carried out with plants in the fourth category, those whose broken chromosome had no knob but likewise no observable deficiency. The selfing of eight plants produced 1,818 *C* non-variegated kernels, 605 *c* kernels, and no variegated kernels. Only one plant was crossed by *c*. This resulted in 112 *C* non-variegated kernels, 139 *c* kernels, and no variegated kernels. Pollen of two plants in this category was placed on silks of *c*-carrying plants. There resulted 904 *C* non-variegated kernels, 883 *c* kernels, and no variegated kernels. Complete healing of the broken end likewise occurred in these plants. These chromosomes, although deficient for the knob, behaved strictly as a normal chromosome 9.

The broken end in plants of the fifth category likewise showed a permanent healing. Since the transmissions of the deficient chromosomes is dependent upon the extent of the deficiency, no ratios will be given here.

The plants in the third category, those whose broken chromosome possessed a duplication of the short arm of chromosome 9, gave entirely different results. Many variegated kernels resulted from the selfing and backcrossing of these plants. It will be shown that this is not due to the instability of the original broken end but to the production at meiosis in these plants of chromosomes 9 with new broken ends. These new broken ends are the cause of the variegation in the endosperms of the progeny. These new broken chromosomes become stable in the embryos of these variegated kernels and remain stable from then on. Although the number of variegated kernels arising from a self or a backcross of a plant carrying a duplication depends upon the particular duplication that is present, a summary of the results from crosses involving all the duplications is illuminating. The progeny from selfing eight plants carrying duplications gave 1,052 *C* non-variegated kernels, 841 *c* kernels, and 152 *C-c* variegated kernels. When the female parent carried the duplication, the backcross gave 397 *C* non-variegated kernels, 450 *c* kernels, and 29 *C-c* variegated kernels. When these plants were used as pollen parents, the backcross gave 179 *C* non-variegated kernels, 641 *c* kernels, and 49 *C-c* variegated kernels. Since pollen grains carrying the duplication do not function readily in competition with those carrying a normal chromosome 9, a deficiency of the *C* class is to be expected in the selfed and male backcross progenies. Among a total of 3,789 kernels resulting from these crosses, 230 were variegated for *C-c*. The variegation was of the type which would be produced by a broken chromosome. This presents an extreme contrast to the results recorded from the previous classes where four *C-c* variegated kernels appeared in a total of 24,305 kernels.

A chromosome 9 with a duplication of all or of only a part of the short arm, such as that illustrated in figure 9, can give rise to new broken chromosomes following crossing over. The method by which crossing over can give rise to broken chromosomes is illustrated in figure 11. In this illustration the plant is considered to be heterozygous for a duplication of the short arm of chromosome 9. In the diagram, the end of the short arm of the normal chromosome 9 terminated in a large knob. No knob has been diagrammed in the duplication chromosome. This constitution has been chosen, since it will apply to all the duplications which will be discussed in sections 4 and 5 of this paper. At meiosis, association of the three homologous segments of the two chromosomes 9 is 2-by-2. Two of these associations are diagrammed (a and b, fig. 11). In a, the duplicated segment is associated with the short arm of the normal chromosome 9. A crossover as indicated would give rise to a dicentric chromatid (c, fig. 11). This dicentric chromatid is the equivalent of two chromosomes 9 fused at the ends of their short arms. In b (fig. 11), the two homologous segments in the duplicated

chromosome 9 are associated. A crossover as indicated would result in the same dicentric chromatid (c, fig. 11). Disjunction of homologous centromeres in anaphase I would result in a first division bridge configuration following the crossover in a. Separation of sister centromeres in anaphase II would give rise to a second division bridge configuration following the crossover in b. In the late anaphase or early telophase of these cells, the dicentric chromatid is broken at some position between the two centromeres. If the break occurred at the position of the arrow (c, fig. 11), each of the two broken chromatids would contain a complete set of genes of chromosome 9. If the break occurred at any other position, a deficient chromatid would enter one nucleus and a chromatid with a duplication would enter the sister nucleus. In this way, chromosomes 9 could be produced with various lengths of duplication and deficiencies of segments of the short arm.

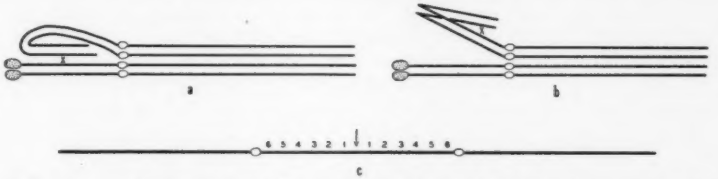

FIGURE 11.—a. Diagram of a meiotic prophase association of a normal chromosome 9 with a large terminal knob and a chromosome 9 with a duplication of the short arm but with no knob. The duplicated segment is homologously associated with the short arm of the normal chromosome 9. A crossover as indicated produces a chromatid composed of two attached chromosomes 9, as shown in c. This will produce a bridge configuration at anaphase I. b. Association of homologous regions of the duplication chromosome 9. A crossover as indicated will give rise to the dicentric chromatid shown in c. A bridge configuration will result in anaphase II.

However, each would possess a newly broken end. Each, therefore, should be capable of inducing variegation in the endosperm tissues to which it is delivered, provided the appropriate genic markers are present to allow detection of variegation. Plants arising from these variegated kernels should show a new series of various types of broken chromosomes 9. All plants with duplications of the type illustrated in figure 9 were heterozygous—that is, contained a duplication chromosome 9 with dominant genes in the duplicated segments and a normal chromosome 9 carrying the recessive alleles. In deriving the constitution of the dicentric chromosome produced following crossing over, it is necessary to insert the knobs and pycnotic regions where they occur in each of the duplicated chromosomes.

Meiotic anaphases were observed in all plants heterozygous for duplications. All showed bridge configurations in some of the anaphase I and II cells. The total percentages and proportions of bridges in the two divisions were not the same and should not be the same for all duplications. A summary of meiotic behavior and the types of progeny will be confined to a

single case, that of duplication 1276-1 (arrow to right in type I, fig. 9). In this plant, 189 microsporocytes in anaphase I were recorded. Nineteen, or ten percent of these showed a bridge configuration. In the second division, recordings were made only when the two sister cells were in mid-anaphase. Among 144 such dyads, 38 or 26 percent showed a bridge configuration in one of the cells of the dyad. This plant was selfed and produced 126 *C* non-variegated kernels, 97 *c* kernels, and 13 *C-c* variegated kernels. At the time

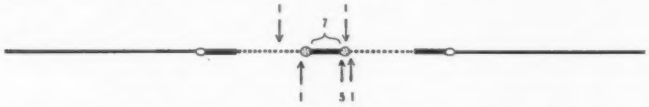

FIGURE 12.—Chromatin constitution of the dicentric chromatid produced by crossing over in plant 1276-1. The constitution of the recovered broken chromosome in plants arising from the variegated kernels may be referred to this dicentric chromatid. The recovered broken chromosomes have the constitutions to the right of the arrows and to the right of positions within the bracket. The numbers given above or below indicate the number of plants in which the broken chromosome had this particular constitution.

of pollination there were no homozygous *c* plants to which it could be crossed. Consequently, it was crossed to a normal plant of the constitution *C c*. This cross produced 187 *C* non-variegated kernels, 144 *c* kernels, and 18 *C-c* variegated kernels.

The chromosome 9 constitution was determined in 14 plants arising from the *C non-variegated* kernels of the self of 1276-1. Thirteen of these plants possessed a normal chromosome 9 and a chromosome 9 with the duplication—that is, had the same constitution as the parent. One plant possessed two normal chromosomes 9, the result of a crossover between the short arm of the normal chromosome 9 and the proximal homologous segment of the duplicated chromosome 9. Cytological determination of the chromosome 9 constitution of eight plants arising from variegated kernels of the self and of nine plants arising from the variegated kernels of the outcross were illuminating. All 17 plants possessed a newly broken chromosome 9. In eight of these plants, derivation from the dicentric chromatid produced by crossing over of the type illustrated in figure 11 was unmistakable. In the parent plant, 1276-1, a short segment of pycnotic region 6 was inserted between the two knobs and thus between the proximal and distal duplicated segments of the short arm (see arrow to right, type I, fig. 9). A crossover as diagrammed in a or b of figure 11 would give rise to a dicentric chromatid with the constitution shown in figure 12. All the newly produced broken chromosomes 9 showing a pycnotic segment extending beyond the knob may be traced to this dicentric chromatid. For illustrative purposes, the chromosome constitution of 16 of the 17 plants arising from variegated kernels may be referred to this dicentric chromatid (fig. 12). The arrows indi-

cate the positions of breakage; the numbers above or below the arrows indicate the number of plants whose broken chromosome 9 constitution corresponded to that part of the dicentric chromatid to the right of the arrow. A new duplication, derived from secondary fusions and breaks, was present in one plant. Its constitution is shown in c, figure 13. The method of deriving the origin from the dicentric chromatid (a and b, fig. 13) is the same as that used in figure 9.

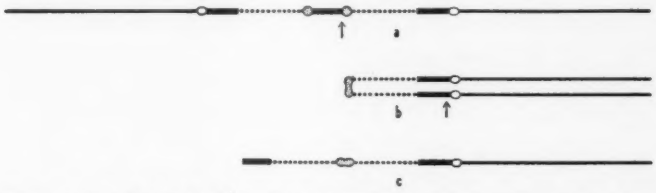

FIGURE 13.—Illustration of the origin of a new duplication chromosome 9 from duplication 1276-1 as a consequence of the breakage-fusion-bridge cycle. a. Dicentric chromatid produced following a crossover in plant 1276-1 (heterozygous for a duplication shown in type I, figure 9). The arrow indicates the position of the first break, the broken chromatid to the right being in the line of descent. b. The two split halves of the broken chromatid fused at the position of previous breakage. The arrow indicates the position of the second recognizable break which gave rise to the recovered broken chromosome with a new duplication shown in c.

The stability of these newly broken chromosomes following their passage through a sporophytic generation is indicated by kernel characters obtained from selfing seven of the plants whose broken chromosome ended either in a knob or in a short pycnotic segment extending beyond the knob. There were 1,385 *C* non-variegated kernels, 456 *c* kernels, and no variegated kernels. The ratio obtained from selfing the plant with the newly derived long duplication, illustrated in figure 13, gave 104 *C* non-variegated kernels, 80 *c* kernels, and 26 *C-c* variegated kernels. These variegated kernels were expected, for the process of forming new broken chromosomes at meiosis is the same in this plant as it was in the parent plant with the original duplication. The selfed ear from the plant with the short duplication (left-most arrow in fig. 12) was badly diseased. However, pollen of this plant placed upon silks of a *C c* plant with two normal chromosomes 9 gave 98 *C* non-variegated kernels, 68 *c* kernels, and three *C-c* variegated kernels. From this plant, likewise, variegated kernels should appear and do appear in the progeny.

Although the original duplicated chromosome in plant 1276-1 carried *C* in each of the homologous segments, it is not certain that this genic constitution will be preserved when this chromosome is passed on to the next generation. Following crossing over at meiosis in plant 1276-1, the *c* gene carried by the normal homologue, could have been inserted into one of the

duplicated segments. As stated above, 13 of the 14 examined plants derived from the *C* non-variegated kernels of the self of 1276-1 possessed the original duplicated chromosome 9 and a normal chromosome 9. Regardless of crossing over, a *C* gene must be present in at least one of the homologous segments of the duplication. Consequently, variegated kernels should appear in the progeny of these plants following appropriate crosses because newly broken chromosomes should be produced during meiosis in these plants in exactly the same manner as occurred in the parent plant. The results of crosses involving these plants are summarized in table 7. It is obvious from this table that variegated kernels do appear. The plants arising from these variegated kernels should show, in turn, newly derived broken chromosomes.

TABLE 7

Character of kernels in the progeny of crosses involving a duplication of the short arm of chromosome 9 derived from plant 1276-1.

CROSS	*C* NON-VARIEGATED KERNELS	*c* KERNELS	*C-c* VARIEGATED KERNELS
Duplication chromosome 9 carrying *C* / Normal chromosome 9 carrying *c* ♀ × *cc*♂	192	242	16
Reciprocal	179	641	49
Duplication chromosome 9 carrying *C* / Normal chromosome 9 carrying *c* selfed	131	90	16

From the numerous examples so far presented in this paper, there can be little doubt that variegation in the endosperm tissues is correlated with the presence of a chromosome with a broken end. Furthermore, if the constitution of chromosome 9 in a plant is known, it is possible to predict whether or not variegated kernels will appear in the progeny. All predictions have been completely verified.

IV. THE CORRELATION OF VARIEGATION AND BROKEN CHROMOSOMES IN THE PROGENY OF A NATURALLY ARISING DUPLICATION IN CHROMOSOME 9

Before the investigations described in section III of this paper had been undertaken, studies were underway on a chromosome 9 possessing a duplication of the short arm. Cultures containing this chromosome gave relatively high percentages of variegated kernels in their progeny. The origin of this duplication was unknown. It was discovered in a genetic strain of maize belonging to DR. L. J. STADLER. The author is indebted to DR. STADLER for use of this duplication in the study now reported. The consti-

tution of the duplicated chromosome 9 is essentially similar to the duplicated chromosome 9 illustrated in type III of figure 9. The duplication included practically all of the short arm of chromosome 9. No knob was present. This duplicated chromosome carried the genes *I* and *Wx* in the two homologous segments. *I*, an inhibitor of aleurone color development, is placed at the same locus as the gene *C* (HUTCHISON 1922). The color pattern of variegation induced by broken chromosomes derived from this duplicated chromosome is the reverse of that described in section III, for loss of the *I* gene from cells of the aleurone allows color (*i*) to appear in these cells.

Since the origin of the broken chromosome at meiosis in plants heterozygous for this duplication is exactly the same as that described in the previ-

TABLE 8

$$\frac{I\ Wx \text{ duplication chromosome } 9}{i\ wx \text{ normal chromosome } 9} \times i\ wx \text{ normal chromosome } 9$$

CROSS	NON-VARIEGATED KERNELS				VARIEGATED KERNELS	
	I Wx	*I wx*	*i Wx*	*i wx*	*I-i Wx-wx*	*I-i wx*
♀ Parent heterozygous	438	0	23	445	17	0
♂ Parent heterozygous	62	0	29	765	27	3

ous section, the evidence obtained from this duplicated chromosome will be but briefly reviewed.

In plants heterozygous for the duplicated chromosome 9,—that is, possessing one duplicated chromosome 9 and a normal chromosome 9,—bridge configurations are seen in both the first and second meiotic mitoses. Of the 193 anaphase I cells recorded, 26 or 13.4 percent showed a bridge configuration. Among 74 dyads in anaphase II, 10 or 13.5 percent showed a bridge configuration in one of the cells of the dyad.

In these plants, the duplication chromosome 9 carried *I* and *Wx* in each of the duplicated segments. No knobs were present in the duplication chromosome 9. The normal homologue carried *i* and *wx*. Its short arm terminated in a large knob. The chromosome 9 constitution of these plants was similar to that shown in figure 11. Following crossing over as diagrammed in figure 11, the order of the genes between the two centromeres of the dicentric chromatid could be *Wx I i wx*, *Wx I I wx*, or *Wx I I Wx*. Following breakage of the dicentric chromosome at a meiotic anaphase, broken chromosomes with various genic compositions should arise. Those possessing *I Wx* or *I wx*, either singly or in duplication, should produce variegation for color if delivered to an endosperm following the cross of this plant to one possessing two normal chromosomes 9 carrying *i wx*.

The results of such a cross are given in table 8. In the first line of the table, the female was the heterozygous parent. In the second line, the male was the heterozygous parent. The low frequency of the *I Wx* non-variegated class in this latter cross is due to the low transmission through the pollen of the chromosome carrying the duplication. Six plants derived from the *I Wx non-variegated* kernels in this latter cross were examined cytologically. Five plants showed the presence of the duplication chromosome 9; one plant possessed two normal chromosomes 9, the short arms of each terminating in a large knob. Since the normal chromosome 9 in all the plant of this study possessed a large terminal knob, the chromosome with *I Wx* in this latter plant obviously arose through a crossover between the normal chromosome 9 and the proximal segment of the duplication chromosome 9. The chromosome 9 constitutions were determined in 26 plants derived from the *I Wx non-variegated* class of the first line of table 8. All 26 possessed the duplicated chromosome 9 and a normal chromosome 9 terminating in a large knob.

The chromosome 9 constitution has been determined for 28 plants derived from the variegated kernels of table 8. A broken chromosome 9 was present in each plant. For illustrative purposes, the type of recovered

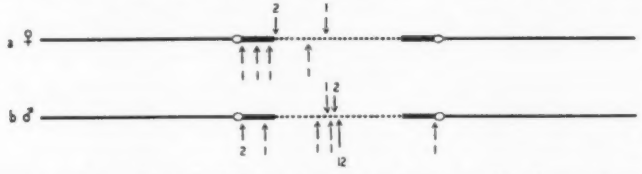

FIGURE 14.—Types of recovered broken chromosomes in plants arising from variegated kernels. The parental chromosome constitution of the heterozygous parent was exactly as diagrammed in figure 11. The dicentric chromatid produced following crossing over is shown in a and b. The constitution of the recovered broken chromosome is that to the right of the arrow in each case. The numbers above or below the arrows indicate the number of plants with this particular broken chromosome. In a, the female parent contributed the broken chromosome. In b, the male parent contributed the broken chromosome.

broken chromosome may be referred to the original dicentric chromatid from which it arose (fig. 14). The broken chromosome in seven of these plants was introduced by the female parent (variegated kernels in line 1 table 8). The broken chromosomes in the remaining 21 plants were introduced by the male parent (variegated kernels in line 2, table 8). Since the proportion of types of recovered broken chromosomes are not comparable in the two crosses, the types received from the female parent are shown in a (fig. 14), those received from the male parent in b (fig. 14). The composition of the broken chromosome is that to the right of the arrow in each case. The numbers above or below each arrow indicate the number of plants

with this particular broken chromosome. Totalling both crosses, 14 of the plants possessed a duplicated segment. These varied in length from a single chromomere in one plant to the full short arm in three plants. There was only one plant with a deficiency (b, fig. 14), but this deficiency included all of the short arm. The remaining 13 plants had a complete chromosome 9 with neither a duplication nor a deficiency. Twelve of these were introduced by the male parent. In these plants, the broken chromosome 9 could readily be distinguished from the normal chromosome 9, since the former possessed no knob whereas the short arm of the latter terminated in a large knob.

The duplications derived from the rearranged chromosome 9, described in f of section III, could be explained only as the result of successive fusions and breaks, as shown in figure 9. However, it could not be determined

TABLE 9

A. $\frac{\text{Normal chromosome 9 carrying } I}{\text{Normal chromosome 9 carrying } i} \times$ normal chromosome 9 carrying *i*

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	3,074	3,153	1
♂ parent heterozygous	2,213	2,129	1

B. $\frac{\text{Broken chromosome carrying } I\text{, no duplication or deficiency}}{\text{Normal chromosome 9 carrying } i} \times$ normal chromosome 9 carrying *i*

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	1,467	1,419	1
♂ parent heterozygous	3,008	3,011	1

C. $\frac{\text{New duplication chromosomes 9 carrying } I}{\text{Normal chromosome 9 carrying } i} \times$ normal chromosome 9 carrying *i*

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	1,822	2,107	49
♂ parent heterozygous	22	164	4

in some cases and was not determined in others whether the duplications illustrated in figure 14 arose directly from the dicentric chromatid or following subsequent fusions and breaks. Distinctive morphological markers such as knobs and pycnotic extensions, illustrated in figure 9, could not be present in these duplications. The presence of small extra segments inserted between the two main duplicated segments could be identified only with considerable difficulty and was not attempted. However, successive

fusions and breaks must have preceded the formation of the deficient chromosome in b of figure 14 (for discussion, see section III d).

At this point, attention should be called to the contrast in the types of broken chromosomes delivered by the sperm and the egg. Most of those delivered by the female gamete have duplications. The majority of those coming from the male parent are without duplication or possess only a very small duplication. A discussion of the possible significance of this will be postponed until more cases have been considered.

Healing of the broken end in the embryo should result in stability of the broken chromosome in successive generations. The broken chromosomes with no duplication or deficiency should behave as a normal chromosome 9. On the other hand, the broken chromosomes with newly derived duplications should give rise to variegated kernels following crossovers similar to those diagrammed in figure 11. The kernel characters obtained from crossing these various plants by those containing normal chromosomes 9 with *i* in each homologue are given in table 9. A, table 9, represents a control cross the heterozygous parent possessing two normal, unbroken chromosomes 9. In B, table 9, the *I* carrying chromosome possessed a broken end, but no duplication or deficiency was present. In C, table 9, the chromosomes carrying *I* possessed the newly derived duplications of figure 14. The results are as expected and require no further explanation.

V. THE CORRELATION OF VARIEGATION AND BROKEN CHROMOSOMES IN THE PROGENY OF A SHORT DUPLICATION DERIVED FROM A LONG DUPLICATION

All the newly derived duplications illustrated in figure 14 gave rise to variegated kernels in their progeny. Plants arising from these variegated kernels should have a newly broken chromosome. This has proved to be true. However, extensive tests were conducted with only one of these duplications, the short duplication composed of a fifth of the short arm (third arrow from left, b, fig. 14). The plant possessing this short duplication carried *I* in the duplicated chromosome and *i* in the normal homologue. When pollen carrying a normal chromosome 9 with *i* was placed upon silks of this plant, there resulted 149 *I* non-variegated kernels, 188 *i* kernels, and two *I-i* variegated kernels. When pollen of this plant was placed on the silks of a normal plant carrying *i*, there resulted seven *I* non-variegated kernels, 92 *i* kernels, and three *I-i* variegated kernels. Twenty-one plants derived from the *I non-variegated* kernels in the former cross were examined for the constitution of the chromosome 9 delivered by the female parent. The short duplication was present in 20 of these plants. In one plant, two normal chromosomes 9, each terminating in a large knob, were present—obviously the result of a crossover which inserted *I* into the normal chromosome 9 of

the female parent. Plants derived from the seven *I non-variegated* kernels in the latter cross were likewise examined. Five of these had received the short duplication chromosome 9, and two had received a normal chromosome 9—the consequence of a crossover in the parent plant.

The formation of dicentric chromatids at meiosis has been assumed to be the means of producing the broken chromosomes which give rise to variegation. The method of origin of the dicentric chromatids is the same as that diagrammed in figure 11. To verify this, meiotic anaphases were examined in plants heterozygous for the short duplication. Among 231 anaphase I configurations recorded, 20 or 8.6 percent showed a bridge configuration. At anaphase II, 150 dyads were recorded. Eleven or 7.3 percent showed a bridge configuration in one of the cells of the dyad. Plants derived from the *I Wx* non-variegated kernels in the crosses given above which were heterozygous for the duplication (carrying *I* in the duplicated chromosome and *i* in the normal chromosome) were crossed with plants possessing two normal chromosomes 9 each carrying *i*. The results are shown in table 10. These crosses produced a total of 151 variegated kernels.

TABLE 10

Types of kernels resulting from the cross.

$$\frac{\text{Short duplication chromosome 9 carrying } I}{\text{Normal chromosome 9 carrying } i} \times \text{normal chromosome 9 carrying } i$$

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	6,684	6,774	106
♂ parent heterozygous	698	3,081	45

In contrast, the three examined F_1 plants with two normal chromosomes 9, which were derived from the *I Wx* non-variegated kernels, produce no variegated kernels following similar crosses.

A cytological determination was made of the chromosome 9 constitution of 15 plants derived from the variegated kernels of table 10 to verify the supposition of the presence in these plants of a newly derived broken chromosome. This proved to be true. The constitutions of the newly derived broken chromosomes are illustrated in figure 15. The method of illustration is the same as that used in figure 14. The derived broken chromosome is referred to the dicentric chromatid from which it arose. The broken chromosomes have the constitutions to the right of the arrows. Below each arrow is given the number of plants having this particular composition of its broken chromosome; in a, the female parent contributed the broken chromosome; in b, the male parent contributed the broken chromosome. Five

of the 15 plants had a newly derived broken chromosome with no duplication or deficiency. Nine plants possessed newly derived duplications of various lengths, and one plant possessed a broken chromosome deficient for a terminal segment which included approximately one-fourth of the short arm.

FIGURE 15.—The description for this figure is similar to that given with figure 14 except that the duplication in the heterozygous parent was short, composed of a terminal segment only one-fifth the length of the short arm. Nevertheless, following crossing over, the dicentric chromatid has the same constitution as that in c of figure 11 and in a and b of figure 14.

As in previous cases, these newly broken chromosomes were tested for their stability in the succeeding generation. The results are given in table 11. They are as expected. The five plants whose broken chromosome had neither a duplication nor a deficiency produced no variegated kernels (A, table 11). In contrast, tests of seven of the newly derived duplications (B,

TABLE 11

Tests of the stability of broken chromosomes derived from the chromosome 9 with the short duplication.

A. $\frac{\text{Broken chromosome 9 carrying } I \text{ (no duplication or deficiency)}}{\text{Normal chromosome 9 carrying } i} \times$ normal chromosome 9 carrying i

TYPE OF CROSS	NON-VARIEGATED KERNELS		VARIEGATED KERNELS
	I	*i*	*I-i*
♀ parent heterozygous	662	661	0
♂ parent heterozygous	1,524	1,501	0

B. $\frac{\text{Newly derived duplication chromosomes 9 carrying } I}{\text{Normal chromosome 9 carrying } i}$ ♀ × normal chromosome 9 carrying i♂

NON-VARIEGATED KERNELS		VARIEGATED KERNELS
I	*i*	*I-i*
956	1,119	20

table 11) gave a total of 20 variegated kernels. Each ear possessed one or more variegated kernels. There can be little doubt that the plants arising from these variegated kernels would again show newly broken chromosomes. In view of the extensive analysis of the chromosome composition of plants derived from variegated kernels in the progenies of (1) the rearranged chromoxome 9 (section III, d), (2) the duplication chromosomes 9 derived from the rearranged chromosome 9 (section III, f), (3) the original long duplication which arose in a genetic culture of maize (section IV), and

and (4) the short duplication derived from this latter duplication (section V), it was not considered necessary to make these tests. The evidence is conclusive from all of these cases that variegation in the endosperm tissues is related to the presence of a chromosome with a broken end, the original break having occurred in the previous meiotic divisions. The type of variegation which appeared in these kernels supports the assumption of the breakage-fusion-bridge cycle as the causal agent for this variegation, that is, the deletions of genes from some nuclei and their duplication in other nuclei.

VI. DISCUSSION

(a) *The positions of the breaks in anaphase bridge configurations in successive nuclear divisions*

In the preceding sections of this paper the determination of the chromosome constitution of 186 plants, derived from variegated kernels, has been given. In 180 of these plants, a chromosome with a broken end was found. In each case, the chromosome with the broken end carried the genes associated with the variegation, these genes being located in the arm of the chromosome which possessed the broken end. Of the six exceptional cases, three resulted from hetero-fertilization; consequently, the chromosome constitution of the plant tissues could not be related to that in the endosperm tissues. The remaining three exceptional cases showed an altered chromosome, but the relation of the alteration to the variegation appearing in the endosperm could be determined in only one case (see section III d). There can be little doubt that the variegation in the endosperm tissues, described in this paper, is related to the presence in this tissue of a newly derived broken chromosome. It is necessary that the broken end be newly derived for a broken end heals when introduced into a zygote and is no longer capable of producing variegation either in the resulting sporophytic tissues or in the gametophytic and endosperm tissues of succeeding generations. This has been clearly established.

In all cases mentioned in this paper, the broken chromosome had its origin in a meiotic mitosis. The variegation was confined to the endosperm tissues in the generation immediately following. It made no difference whether the broken chromosome was introduced through the pollen grain or through the embryo sac. In either case variegation resulted in the endosperm tissues. The character of this variegation was clearly of the type which should arise following the breakage-fusion-bridge cycle where successive breaks do not always occur at positions of previous fusions (see fig. 1). Evidence for the initiation of this cycle has been given in a previous publication (McCLINTOCK 1938b). The observations were confined to the first division of the microspore or male gametophyte. This is the first divi-

sion following the production of the broken end. The evidence for continuation of this cycle in the following gametophyte divisions is obtained inferentially in the study reported here. In several cases the broken chromosome in the endosperm and in the embryo were not alike in chromosome constitution. The difference could readily be accounted for if fusion had occurred between the broken ends of sister chromatids in the generative nucleus followed by an anaphase bridge configuration during the division of this nucleus. If the break in this bridge configuration occurred closer to one centromere than to the other, each sperm would receive a broken chromosome, but the constitution of the broken chromosome in each sperm would differ.

Evidence for the breakage-fusion-bridge cycle in the female gametophyte is likewise inferential. The duplications 1276-1 and 1503-2 (type I, fig. 9) and duplication 1269 (type V, fig. 9) were delivered to the zygote through the female gametophyte. These duplication chromosomes have obviously been derived from a dicentric chromatid in the preceding meiotic mitosis. At least one fusion and break following the original break must have occurred to give rise to duplications 1276-1 and 1503-2. At least two such fusions and breaks must have occurred to have produced the duplication of plant 1269.

On the assumption of the continuation of the breakage-fusion-bridge cycle in the gametophytic tissues, the agreement in so many cases between the genic constitution of the broken chromosome delivered to the endosperm and that delivered to the zygote might appear surprising. Two explanations are possible. First, the break in an anaphase bridge configuration may tend to occur at a median position between the two centromeres. Secondly, following an original meiotic break, the break in the successive anaphase figures may tend to occur at positions of previous fusions. Either or both possibilities must be considered only as trends; otherwise, no duplicated chromosomes would have been produced following breakage of the dicentric chromatid of figure 8 nor would extensive variegation be produced in kernels receiving broken chromosomes.

Evidence obtained from both meiotic anaphase configurations and from examination of broken chromosomes in the prophase of the first microspore division has indicated that the original break in a bridge configuration at a meiotic anaphase may occur at any position between the two centromeres. However, no data have been obtained which could be treated statistically. It is certain, though, that breaks need not occur at median positions. This is confirmed by the number of cases where the recovered broken chromosomes possessed long duplications or deficiencies.The evidence does suggest, however, that following an original break, the breaks in the succeeding anaphases may tend to occur at positions of previous fusions. This is

based on three sets of evidence. The broken chromosomes arising from the dicentric chromatid of figure 8 (see table 4) may be considered first. A functional pollen grain could arise only when the original meiotic break occurred either immediately to the right of the inner small knob or at a position between this point and the left centromere. Thus, a broken chromosome can be transmitted through the pollen only after a decidedly nonmedian break in the original dicentric chromatid. All other breaks would result in broken chromatids with deficiencies. A pollen grain whose tube nucleus does not have a complete set of genes of chromosome 9 is not functional. In the summary table 5 the percentage is given of the various types of broken chromosomes received by the embryos. Eleven and one-tenth percent possessed a deficient chromosome 9, and 11.1 percent possessed a duplication of chromosome 9. Each of these types of chromosomes could be contributed by the pollen only following a secondary break which did not occur at the position of previous fusion. Thus, it is established that in approximately one-fourth of these cases, a secondary break did not occur at the position of previous fusion. The broken chromosomes transmitted by the female gamete cannot be so analyzed, since deficiencies of approximately one-third of the distal part of the short arm can produce functional embryo sacs (McCLINTOCK unpublished). The chromosomes with duplications are the only ones which must have originated following a successive break which was not at the position of previous fusion. The high percentage of cases where the broken chromosome ended either in the knob or at positions immediately adjacent to it strongly suggests that at anaphase this substance is more readily broken than are other parts of the chromosome. This introduced an obstacle in the analysis of the randomness of the breaks. However, most of the recorded broken chromosomes gave no evidence of having undergone the breakage-fusion-bridge cycle, which suggests that breakage in successive anaphases may tend to occur at positions of previous fusions.

The evidence is likewise suggestive when the recovered broken chromosomes illustrated in figures 14 and 15 are analyzed. A surprisingly high percentage (47 percent) of the broken chromosomes delivered to the zygote by the pollen parent had neither a duplication nor a deficiency. Although the number of chromosomes examined is small, the figure for this one class is very high. The relative percentages of types of recovered broken chromosomes delivered by the male and female gametes, respectively, are as follows:

	Male gametes	Female gametes
No duplication or deficiency	47 percent	20 percent
Deficiency	6.2 percent	0.0 percent
Duplication	47 percent	80 percent

Pollen grains whose tube nucleus possesses a deficiency do not function. Therefore, chromosomes with deficiencies introduced by the male parent must have resulted from a break which was not at the position of previous fusion. Again, pollen grains whose tube nucleus contains a duplication do not function as successfully as those containing a normal genic complement. Since some of these grains are known to function, it cannot be determined whether the duplications delivered through the pollen arose at the time of meiosis or at a later stage following a break which did not occur at a position of previous fusion. More probably, both factors were involved to give the 47 percent of such cases. When the tube nucleus contains neither a duplication nor a deficiency, the pollen grain functions normally. Pollen grains whose tube nucleus contains a broken chromosome with this constitution would not meet competition. If breaks tend to occur at positions of previous fusions, the constitution of the sperm nuclei and that of the tube nucleus would tend to be similar in many grains carrying broken chromosomes. Among all the pollen grains containing broken chromosomes produced by these plants, those whose tube nucleus contains neither a duplication nor a deficiency would be selectively favored in effecting fertilization. If there were a tendency for breaks to occur at positions of previous fusions, a high percentage of the plants receiving broken chromosomes through the pollen would have neither a duplication nor a deficiency in the broken chromosome. Although the numbers are small, the results so far obtained are very suggestive of this interpretation. Since selection against megaspores and embryo sacs with duplications and in some cases small deficiencies does not occur, no such proportionality of recovered broken chromosomes should appear. The numbers of examined cases are too few to be decisive, but the percentages of types of broken chromosomes recovered from the female gametophyte does not appear to be the same as the percentages recovered from the male gametophyte.

The third line of evidence relates to the simplicity of the recovered broken chromosomes with duplications. Two divisions in the male gametophyte and possibly the first division in the zygote should follow the breakage-fusion-bridge cycle. If the microspore nucleus possessed a broken chromosome with at least a full set of genes, a decidedly non-median break in the bridge configuration at the first microspore division in most cases would result in a tube nucleus with a deficiency or a duplication. Selection against the functioning of such grains could result. However, no such effect should follow the breaks in the next divisions in the male gametophyte (or in the first division of the zygote if a bridge configuration occurs here). Since some functional pollen grains should have had duplications in their generative nuclei, repeat duplications could be produced following a non-median break in the anaphase of the division of the generative nucleus.

However, such repeat duplications have not been observed. In the female gametophyte, three divisions separate the megaspore from the egg nucleus. Since there is no selection against megaspores or embryo sacs carrying duplicated segments in chromosome 9, repeat duplications could be present in the egg nucleus following non-median breaks in the bridge configurations of the division leading to the formation of the egg nucleus. Nevertheless, repeat duplications are rare; only one such case was recognized (type V, fig. 9). Considering the facts given above, it seems reasonable to conclude that there is a tendency for the breaks to occur in the anaphase bridges at the positions of previous fusions, although it has been definitely proven that this does not occur in all such bridge configurations. Since the mechanism which results in fusions of broken ends of sister chromatids is not known, it is not possible to ascribe this breakage at the position of previous fusion to a weak or imperfect union caused by a partial healing of the broken end. The broken end must remain capable of fusion; otherwise variegation would not be produced in the endosperm tissues. This variegation can result only when the breaks do not occur at positions of previous fusions. Furthermore, once a complete fusion has occurred, it must be as strong as that between any other regions in the chromosome; otherwise the broken chromosomes derived from the dicentric chromatid of figures 12 and 13 would tend to be alike. They are not alike, however.

(b) *The type of variegation in endosperms receiving one broken chromosome contrasted with those receiving two broken chromosomes*

The presence of a single broken chromosome in the endosperm tissues was considered in the interpretation of the cause of variegation, as illustrated in figure 1. This chromosome carried the dominant genes. The endosperm tissues are 3n; two sets of chromosomes are contributed by the female gametophyte, and one set is contributed by the male gametophyte. Variegation in the endosperm results when the broken chromosomes are contributed by either the female or the male gametophytes. In the latter case, only one broken chromosome is present. In the former case, two broken chromosomes are present. The interpretation of variegation on the basis of fusion of broken ends of sister chromatids following breakage of an anaphase bridge configuration in the previous division is the only logical assumption when a single broken chromosome is present (contributed by the male parent). However, when two broken chromosomes are present (contributed by the female parent), two possibilities may occur. Either fusion of broken ends could occur between the sister halves of each broken chromosome, following breakage of the anaphase bridge configurations, or the two broken ends of each chromosome could fuse with one another. In the first assumption, each broken chromosome would produce a bridge

configuration in successive mitoses. In the latter assumption, bridge configurations would result when the two centromeres of the dicentric chromatids passed to opposite poles. Interlocking of chromatids resulting in breakage could likewise follow the passage to the same pole of the two centromeres of a chromatid. However, either of the above interpretations would lead to variegation in the endosperm tissues.

If fusions of sister chromatids occurred, the amount of recessive tissue appearing in the variegated kernels should be considerably less when two broken chromosomes are present than when one broken chromosome is present. The dominant genes from two chromosomes would have to be lost to a nucleus before the recessive character would show in the former case, whereas loss of the dominant gene from only one chromosome is necessary in the latter case. Comparative examinations of variegated kernels have not shown this difference. Although the amount of recessive tissue exhibited by individual kernels on the same ear is variable in either case, taken as a whole, the endosperms which receive two broken chromosomes do not show considerably less recessive tissue than those receiving but one broken chromosome. There is a decided difference, however, in the range of color of the various *C* spots in the two cases. The endosperms which receive two broken chromosomes carrying *C* have many more spots of extreme deep color than those which receive but one broken chromosome with *C*. This would occur if many *C* genes were included in some of the nuclei following breakage and fusion of two broken chromosomes. The more *C* genes present, the deeper the color produced. The type of variegation and the more extreme range in color of the *C* spots may be explained as the consequence of fusions between the broken ends of the two chromosomes followed by breaks in each of the dicentric chromatids at similar positions in any one anaphase spindle. That fusions may occur in sporophytic tissues between two broken ends of chromosomes rather than between the two sister halves at the position of previous breakage has been demonstrated in maize where ring-shaped chromosomes were present (McCLINTOCK 1938a). It may likewise occur in the endosperm tissue. Thus, the fusion of broken ends of sister chromatids occur in successive divisions when a single broken chromosome has been introduced in the endosperm, whereas the fusion of broken ends of two chromosomes may occur when two such broken chromosomes are present in the nuclei of the endosperm cells.

(c) *The healing of broken ends of chromosomes*

It has been repeatedly emphasized that the broken end of a chromosome becomes healed in the sporophytic tissues. The breakage-fusion-bridge cycle, which characterizes its behavior in the gametophytic and endosperm

tissues, ceases. The healing is permanent. When this chromosome is reintroduced into endosperm tissues in the following generations, no fusions occur at the broken end between the two sister halves. Furthermore, when two such broken chromosomes are brought together after each has passed through a sporophytic generation, no fusions occur between the two broken ends. No obvious explanation is available for the healing of the broken ends in the sporophytic tissues as contrasted with the lack of healing in the gametophytic and endosperm tissues. The question might arise as to whether the broken end ever heals in the gametophyte or endosperm tissues. Although this may occur in some cases, the evidence suggests that it must be rare. It will be recalled that the majority of variegated kernels arising from the rearranged chromosome 9 (see table 1) had the constitution *C Sh wx*. There were some non-variegated kernels with this constitution. It was assumed that the variegated kernels had broken chromosomes and the non-variegated kernels, non-broken chromosomes. If healing of broken ends occurs, the plants arising from some of the non-variegated *C Sh wx* kernels should have shown a broken chromosome. However, as table 6 shows, none of the plants arising from the *C Sh wx*, non-variegated kernels possessed a broken chromosome. Under certain physiological conditions it is possible that the broken end might heal in the endosperm tissues, but at present these conditions are not known.

Realization of the healing of the broken end in the sporophytic tissues came as a surprise. Evidence from the behavior of ring-shaped chromosomes had indicated that fusions of broken ends of *chromosomes* occurred in the sporophytic tissues when the break originated in this tissue. Although extensively looked for, no evidence for healing of these broken ends was obtained in the ring-chromosome material. Since it has been proven that broken ends can heal, it could be expected that healing of the broken ends arising from the breakage of a ring chromosome might occur under certain physiological conditions.

The broken chromosomes described in this paper and the breaks in the ring-shaped chromosomes all originated as the consequence of mechanical rupture in an anaphase or telophase spindle figure. Breakage induced by other means may not lead to similar consequences—that is, the fusions of broken ends. MULLER (1940) recently reviewed the evidence of the effect of X-rays on the production and behavior of broken ends of chromosomes and concluded that X-ray-induced breakage is followed by 2-by-2 fusions of broken ends, either between parts of the same chromosome, between two chromosomes, or between sister halves of a single chromosome at the position of previous breakage. However, SAX (1940)

and SWANSON (1940) in recent studies on the types of chromosomal abnormalities induced by X-radiation have reported observations which suggest that not all the induced breaks are followed by fusions of broken ends. Their observations were of the division immediately following the treatment of the microspore nucleus and of the generative nucleus of the pollen grain in Tradescantia. It was not shown whether or not these unfused broken ends healed permanently, since the behavior in successive divisions was not followed. With regard to fusions of broken ends, the breaks induced by X-rays and by mechanical rupture apparently are similar. However, the breaks induced in chromosomes by ultraviolet irradiation do not appear to be similar to those produced by X-rays or mechanical rupture. SWANSON (1940) has observed the direct effect of ultraviolet radiation on the breakage of chromosomes. His observations suggest that the breaks induced by the radiation are not followed by fusions of broken ends of chromosomes. Previous to this work, the genetic and cytological evidence of the effect of ultraviolet radiation of maize pollen (SINGLETON 1939, SINGLETON and CLARK 1940, STADLER and SPRAGUE 1936, STADLER 1939) had indicated that the breakage induced by ultraviolet radiation differed from that induced by X-radiation. Most of the recovered chromosomal abnormalities appeared to be terminal deficiencies. The broken end was completely healed. When two such broken chromosomes were present in a single nucleus, no fusion had occurred between them.

Further evidence for the non-fusibility of broken ends produced as the consequence of ultraviolet radiation is suggested by the character of the endosperm tissues which receive a nucleus derived from irradiated pollen. Studies of the sporophytic tissues mentioned above would suggest that many of the endosperm nuclei were receiving chromosomes with broken ends following ultraviolet treatment of pollen. If fusions occur between sister halves of this chromosome at the position of breakage, variegation kernels of the type described in this paper would be expected. These were not present in numbers greater than might be expected from normal, untreated material. If the endosperm possesses a broken chromosome, fusions do not occur at the position of breakage. It becomes apparent that the capacity of a broken end to fuse depends upon the method by which the chromosome becomes broken and the conditions of the cell following the breakage.

Since it has been demonstrated that broken ends of chromosomes under certain conditions retain their capacity to fuse with one another but lose this capacity permanently under other conditions, it will be necessary in the future to determine the nature of these conditions by experimental methods before an understanding can be attained of the factors responsible for fusions or for healing of broken ends.

(d) *The behavior of chromosomes initially broken in the endosperm and in the sporophytic tissues*

In this paper, the variegation in the endosperm tissues has been brought about by the introduction into this tissue of a chromosome which was initially broken in the previous meiotic divisions. The recent evidence reported by CLARK and COPELAND (1940) suggests that the breakage-fusion-bridge cycle will follow if a chromosome receives its initial break after its introduction into the endosperm. The authors examined cytologically the mitotic divisions in the endosperm of strains of maize which had consistently given high percentages of kernels with variegated sectors in the endosperm tissues. The nature of the variegation has been intensively studied by JONES (1937), who came to the conclusion that spontaneous translocations were occurring at a relatively high rate in this material. Such translocations should produce dicentric chromosomes in some nuclei. Breakage of a dicentric chromosome in a succeeding mitotic anaphase would introduce a chromosome with a broken end into the telophase nucleus. If fusions of sister chromatids at the positions of breakage resulted, the breakage-fusion-bridge cycle would be initiated. Extensive cytological examination of mitotic anaphases in this material should reveal bridge configurations many of which should not show an accompanying fragment. The authors have found this to be true and have concluded that the breakage-fusion-bridge cycle will follow a break initiated in the endosperm tissue itself. In view of the healing which results when a broken chromosome is introduced by a gamete into sporophytic tissue, a similar type of investigation needs to be conducted with sporophytic tissues to establish whether or not such healing of a single broken end will occur in this tissue if the break in the chromosome originates in the sporophytic tissue itself. SAX (1940) states that he has obtained evidence for the breakage-fusion-bridge cycle following a breakage of a chromosome in the sporophytic tissues, but a detailed description of the evidence has not been reported as yet.

(e) *Selective orientation of broken chromatids in the second meiotic mitosis*

The high percentage of bridge configurations (32 percent) observed at the first meiotic anaphase in microsporocytes of plants heterozygous for the rearranged chromosome would lead one to expect either a high percentage of variegated kernels on the ears of such plants in the crosses outlined or, if most of the broken chromosomes were highly deficient, a recognizable amount of ovule sterility due to lack of development of ovules whose functional megaspores carried such a deficient chromosome. Neither of these conditions was observed. The percentage of variegated kernels was

low, and there was no marked increase in the sterility of the ovules. Observations of normal maize plants has shown that it is the lower magaspore which develops into the embryo sac (WEATHERWAX 1919). Three explanations may be suggested for this lack of ovule sterility. First, there may be very much less crossing over in the megasporocytes. RHOADES (1940) has reported a difference in crossing over in mega- and microsporocytes in maize for genes of chromosome 5. Nevertheless, a decrease in crossing over would not fully account for the lack of ovule sterility. One could suggest as a second alternative a tendency for selection of megaspores carrying a normal chromosome 9. A third possibility seems more likely. As shown previously, most of the bridge configurations occur at anaphase I. The anaphase bridge delays the migration of the chromatids toward the two poles. Following breakage, each of the two broken chromatids may be held closer to the cell plate than the two normal chromatids. If this orientation is maintained through interkinesis, a broken chromatid would enter each of the two middle megaspores. and a normal chromatid would enter the two end megaspores. The innermost megaspore of the row of four, which normally develops the embryo sac, would then contain a normal chromosome 9. Evidence given in a previous publication (McCLINTOCK 1938b) would lead one to expect this orientation of broken chromosomes in some but not all of the second division spindle figures. This interpretation is similar to that presented by STURTEVANT and BEADLE (1936) as an explanation of low egg sterility in Drosophila in individuals heterozygous for an inversion in the X chromosome. This third alternative is favored as the probable explanation of lack of ovule sterility and is supported by similar evidence obtained from plants heterozygous for an inversion (McCLINTOCK unpublished).

SUMMARY

By use of (1) a rearranged chromosome 9, (2) a duplication arising from this rearrangement, (3) a deficiency derived from this rearrangement, (4) a duplication occurring in a genetic strain of maize, and (5) new duplications derived from this latter duplication it was possible to obtain functional gametes carrying a chromosome 9 whose short arm terminated in a broken end.

In all cases, the broken end arose following crossing over at meiosis which produced a dicentric chromatid. Rupture of the dicentric chromatid at a meiotic anaphase produced the broken end.

During the following gametophytic division, fusions occurred at the position of breakage between the two sister halves of the broken chromatid, resulting in an anaphase bridge configuration. Rupture of this bridge at late anaphase or early telophase again introduced a broken chromosome

into the sister telophase nuclei. This breakage-fusion-bridge cycle continued in the successive gametophytic divisions.

When such a chromosome, initially broken in the previous meiotic mitosis, is introduced into the endosperm tissues of the following generation through either the male or the female gametophyte, the evidence indicates that the breakage-fusion-bridge cycle continues in each successive division. When this chromosome carries dominant genes in the arm with the broken end and when the normal homologue carries the recessive alleles, variegation for these genes appears in the endosperm tissues. This is caused by non-median breaks in the bridge configurations in many anaphase figures which deletes dominant genes from one telophase nucleus and duplicates them in the sister telophase nucleus.

When such a broken chromosome is introduced into the zygote, the broken end heals, discontinuing the breakage-fusion-bridge cycle. This healing is permanent. The broken end behaves in every respect like a normal end. When a broken chromosome has passed through a sporophytic generation, it no longer is capable of producing variegation in the endosperm of the following generation. When two such chromosomes are brought together after each has passed through a sporophytic generation, no fusions occur between their broken ends.

Thus, the breakage-fusion-bridge cycle occurs only in the nuclear divisions of the gametophytic and endosperm tissues when the broken end is newly derived and has not passed through a sporophytic generation.

Evidence is presented which suggests that following an initial meiotic anaphase break, the breaks in the successive anaphase bridge configurations in the following gametophytic divisions tend to occur at the position of previous fusion, but many breaks occur at other positions.

Once a complete fusion has occurred at the position of breakage between the sister halves of a broken chromosome, the fusion results in a union which is as permanent and strong as that between other parts of the chromosome.

The factors responsible for fusions of broken ends or for the healing of a broken end are not understood but are probably related to the method by which the chromosome becomes broken and to the physiological conditions surrounding the broken end.

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